RESEARCH TO DETERMINE THE ACCUMULATION OF ORGANIC CONSTITUENTS AND HEAVY METALS FROM PETROLEUM-IMPACTED SEDIMENTS BY MARINE DETRITIVORES OF THE ALASKAN OUTER CONTINENTAL SHELF

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ABSTRACT

The major objective of this research program was to investigate the fate of petroleum hydrocarbons in the water column and intertidal zone and their bioavailability to, and effects on, the biota. Investigations included two field habitats to supplement laboratory observations. We have learned: 1) That the molecular weight of petroleum hydrocarbon compounds is positively correlated with retention in: (a) weathered oil, (b) oil-impacted sediment, and (c) molluscan tissue; 2) That there was no evidence that the presence of oil enhances the bioavailability of heavy metals to clams (Macoma); 3) That metabolized aromatic hydrocarbons were not found in Macoma tissue, but these polar compounds were present in interstitial water; 4) That petroleum hydrocarbon pollution depresses the feeding rate of marine worms (Abarenicola); and, 5) That pollution is less stressful to a filter feeding clam than to a deposit feeding clam. The work has advanced the level of knowledge by demonstrating that exposure to petroleum hydrocarbons in sediments can result in accumulation and retention of heavier polynuclear aromatic hydrocarbons, but does not enhance uptake of trace metals. These findings have been described in Quarterly Reports and Annual Reports to NOAA/OCSEAP and several journal articles are published, in press, or submitted.

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I. EXECUTIVE SUMMARY

1. EXECUTIVE SUMMARY

Substantial proportions of the intertidal zone of the lower Cook Inlet shoreline consist of fine-grained muddy sediments. Sites with significant areas of such sediment include: Koyuktolik Bay, China Poot Bay, the north shore of Kachemak Bay, the north side of Homer spit, the northwest coast of the Kenai Peninsula, Tuxedni Bay, Chinitna Bay, Iniskin Bay, Bruin Bay, and McNeill Cove (Sears and Zimmerman, 1977). Petroleum or its products which impact muddy sediments as a result of tanker accidents, platform spills, or chronic leakages may persist and affect local organisms for periods of years (Mayo et al., 1978; Teal et al., 1978, Krebs and Burns, 1977; Sabo and Stegeman, 1977). These effects are of more than local interest. Dames and Moore (1979) conducted ecological studies of the intertidal habitats of lower Cook Inlet and concluded, with respect to the mudflats, that "fish, crabs, and ducks move onto the intertidal flats during high tides, and the shorebirds move in during low tides. Commercially, the most important of these interactions appears to be that of juvenile salmon. The consequence is that a very large proportion of the tissue produced on the flats is exploited by predators from other systems. This is particularly important on the west side of the inlet because of 1) the richness of the mud flats, 2) the large proportion of mud flat habitat in the intertidal zone and, 3) the potential susceptibility of this assemblage to oil pollution."

In view of the significance and vulnerability of this habitat type in lower Cook Inlet a series of investigations has been carried out to determine the extent to which populations of invertebrates might become contaminated by specific components of petroleum hydrocarbons. The effects of crude oil on some measures of the growth, activity, and well-being of the intertidal populations, as well as the effect of weathering on the oil itself have also been examined.

The species of organisms used in these investigations were selected to represent genera and feeding types which are important in the habitats under consideration. They included Macoma inquinata, a species very closely related to Macoma balthica which is among the most abundant bivalves in the

mud flat assemblage at Glacier Spit, Chinitna Bay, studied by Dames and Moore (1979). These clams are surface deposit feeders. Another clam, Protothaca staminea, was chosen to represent the suspension feeders, such as Mya which are also present in large numbers in the Glacier Spit fauna. The polychaete, Abarenicola pacifica represents another phylum and another nutritional type, namely non-selective deposit feeding. It is found in large numbers in the upper intertidal zone of Glacier Spit and in smaller numbers in sandier sites of Deep Creek and Homer Spit in lower Cook Inlet.

The toxicity of petroleum hydrocarbons is correlated with their content of aromatic compounds. Furthermore, concern has been expressed that some polynuclear aromatics, or their metabolizes, which are carcinogenic might be taken up from oil-impregnated sediment by invertebrates and transferred to higher trophic levels in the food chain. The accumulation of specific aromatic compounds from contaminated sediment was therefore investigated. In the case of the surface deposit feeding clam Macoma there was a positive correlation between molecular size and accumulation. The two-ring naphthalenes and the three-ring compound phenanthrene were not accumulated to concentrations above those in the sediment after sixty days of exposure. The average accumulation of two and three ring aromatics by the suspension-feeding clam Protothaca was even lower than in the deposit feeder.

By contrast the four-ring compound chrysene and the five-ring benzo(a)-pyrene, which are potential carcinogens or metabolic precursors of carcinogens, continued to be accumulated over a sixty day period by Macoma, tissue magnification factor for the two compounds were 11.7 and 5.3, respectively, (wet weight tissue per wet weight sediment). Abarenicol a accumulated and retained chrysene initially, but reached a plateau at a concentration some six times as high as that in the sediment after fifteen days. Phenanthrene and benzo(a)pyrene were also accumulated for fifteen days to levels four and six times as high, respectively, as those in the sediment. But in the polychaete the concentrations of these compounds fell to 53% and 68% of their peak values over the next 45 days, though the sediment concentrations remained high. No carcinogenic metabolizes were found to accumulate in the tissues of either Macoma or Abarenicola. These data may be useful in estimating the degree of risk from this source to higher organisms at specific levels of sediment contamination.

Concern has also been expressed that the presence of petroleum hydrocarbons in sediment might, by altering either the physical relations of heavy metals to the sediment or the physiology of invertebrate organisms, result in an increased rate of uptake and retention of the metals by the organisms. However, in several experiments in which they were fed with crude oil contaminated detritus for up to fifteen days Macoma showed no tendency to take in more heavy metals than controls did.

The experiments just mentioned did indicate that Macoma took in less food when the food was covered with oil. This finding is consistent with the fact that the condition index, a measure of nutritional status of bivalves, declined by 16% in Macoma exposed in the field for 38 days in summer to sediment containing 360 ppm crude oil. Another index which has been proposed as a measure of well-being in bivalves is the ratio of taurine to glycine in the intracellular free amino acid pool (Bayne, et al., 1976; Jeffries, 1972). This ratio declined from 0.89 to 0.55 in the oil-exposed Macoma giving further evidence for their stressed condition. When exposed to oil under similar conditions in winter 83% of the Macoma died.

By contrast, when the suspension-feeding clam Protothaca was exposed to 850 ppm oil in sediment 85% survived, their average condition index declined by only 6%, and their taurine:glycine ratio increased. It appears that suspension feeding clams are less susceptible to damage from oil in sediment than are deposit feeding clams. The significance of this finding for areas of fine-grained sediment in lower Cook Inlet lies in the fact that the two most abundant genera of bivalves are the deposit feeding Macoma and the suspension feeding Mya. There is therefore a potential for differential mortality or effects on growth and wellbeing on these two groups by any oil pollution incident, with subsequent effects on the patterns of populations and resource utilization in these habitats.

The behavior of organisms is often a sensitive indicator of environmental change. One form of behavior of burrowing animals such as Abarenicola which can be quantitatively measured is the deposition of fecal casts on the surface which reflects the amount of sediment being swallowed. When exposed to sediment containing 1000 ppm or 250 ppm crude oil in artificial burrows in the laboratory their deposition rate was reduced by 70% and 36% respectively. In the field all Abarenicola exposed to 1000 ppm died, and those exposed to 200 ppm reduced their burrowing rate by 45%. This reduction however was transient, and after

a week these animals were turning over as much sediment as the controls. It seems probable that, at some concentrations, oil in sediment will reduce the amount of material that is transported from subsurface sediment to the surface, which in turn would reduce the rate of chemical and/or microbial degradation of the oil.

The next phase of this investigation dealt with changes in the composition and properties of petroleum which might be anticipated after a spill. Crude oil was layered over sea water and weathered under different conditions of sunlight and agitation. Monoaromatic aromatic hydrocarbons, a toxic fraction, and shorter straight-chain saturates (C_8 to C_{10}) disappeared in 24 days under all conditions used. Simulation of violent agitation by spraying water on the oil resulted in an increased loss of other relatively light fractions, which led to a relative increase in the concentration of three ring aromatic compounds, the heaviest examined. Without violent agitation, only 3,6-dimethyl phenanthrene increased in relative concentration. The presence or absence of direct sunlight had no significant consistent effect on the composition of the oil. The implication of these findings are that oil spilled during periods of storms would be poorer in low molecular weight fractions, probably reducing its immediate toxicity, but would provide relatively more heavy compounds with a greater probability of accumulation from sediment. Photoperiod as such would have less effect.

Finally, a study has been made of the fate of those polynuclear aromatic hydrocarbons that become mixed into the intertidal sediment. The most complete data are available for the fate of phenanthrene in a silty sand, containing 28.2% particles smaller than 50 microns in diameter. In this substrate 70% of the added ^{14}C-labelled phenanthrene was still present after sixty days in a simulated intertidal environment. Less than 0.2% of the radioactivity in the sediment was found in the interstitial water at any time, and 75% to 100% of this was present as $^{14}CO_2$. All of the chrysene and 75% of the benzo(a)pyrene mixed into this substrate remained in place after 60 days. In a somewhat coarser substrate, containing fewer than 4% particles smaller than 50 microns, 77%, 67%, and 8% respectively of added benzo(a)pyrene, chrysene and phenanthrene remained after sixty days. As much as 3% of the ^{14}C-label added or phenanthrene appeared in the interstitial water. It is apparent that the heavier the aromatic compound and the finer the grain size the longer is the residence time in sediment. In the

case of phenanthrene, loss from the sediment results at least partially from microbial breakdown of the hydrocarbon to carbon dioxide.

The investigations carried out under this research unit have resulted in a series of publications, and manuscripts submitted for publication. These works and summaries of results of other efforts, which are presented in the following sections of this report, include the details of the procedures and results of the studies.

II. BIOAVAILABILITY OF PETROLEUM HYDROCARBONS FROM SEDIMENT

II-A:

THE FATE OF POLYAROMATIC HYDROCARBONS IN AN INTERTIDAL SEDIMENT EXPOSURE SYSTEM: BIOAVAILABILITY TO MACOMA INQUINATA (MOLLUSCA:PELECYPODA)

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ABSTRACT

Macoma inquinata, a deposit-feeding clam, was exposed for 60 days to sediment containing ^{14}C-labelled phenanthrene, chrysene, or benzo(a)pyrene. Over 70% of the chrysene and benzo(a)pyrene, but only 8% of the phenanthrene, remained in the sediment. The concentrations of chrysene and benzo(a)pyrene in the clams rose steadily, reaching levels 11.6 and 5.2 times as high as those in the sediment. The tissue phenanthrene concentration rose for 3 days, then fell to 1/8 of the initial concentration. No polar metabolizes of any of the hydrocarbons were detected in sediment or tissue.

INTRODUCTION

When petroleum or its products are mixed into marine intertidal sediments the heavier aromatic compounds may remain for years (Burns and Teal, 1979]. Concern has been expressed that these compounds, some of which are carcinogens or metabolic precursors of carcinogens, may accumulate in the tissues of organisms living in impacted sediments, undergo biomagnification, and be transferred along the food chain, becoming hazardous to the ultimate consumers, including man. A study has therefore been undertaken to investigate the duration of retention of 3-, 4-, and 5-ring polynuclear aromatic hydrocarbons (PAH) in sediment under simulated intertidal conditions and the uptake of these compounds by invertebrate organisms. The distribution of the compounds between sediment particles and interstitial water and the presence of polar metabolizes of the PAH were also studied. The first paper of this series describes the fate of phenanthrene, chrysene, and benzo(a)pyrene in a moderately coarse substrate containing the clam Macoma inquinata Deshayes, 1855.

MATERIALS AND METHODS

Exposure system

9-^{14}C phenanthrene (11.3 m Ci/mmol), 5, 6, (11, 12)-^{14}C chrysene (6.3 m Ci/mmol), and 7, 10-^{14}C benzo(a)pyrene (25.3 m Ci/mmol) were purchased from Amersham-Searle Co., Arlington Heights, Illinois. Impurities associated with benzo(a)pyrene degradation have been characterized by reverse phase high pressure liquid chromatography (Clarke, 1976). We have recently used this technique to determine the radio-purity of all three substrates used in these studies. Phenanthrene and chrysene undergo no significant chemical degradation under prolonged storage. Benzo(a)pyrene, however, showed the presence of about 10% impurities and, therefore, was purified by silica gel chromatography

immediately before exposure to assure that no degradative chemical artifacts were introduced to invalidate the radioactivity analyses. The purification procedure was: benzo(a)pyrene (25.3 mCi/mmol, 200 µCi in 1 ml benzene} was chromatographed over 10 grams of silica gel (Grace Davison Chemical Co., 100/200 mesh - heated overnight at 120°C) using benzene as the eluent. Ten milliliter fractions were collected and pure benzo(a)pyrene eluted from the column in the second 10 ml fraction. The column and collection tubes were wrapped in aluminum foil to minimize the exposure of the benzo(a)pyrene to light. The benzene was removed under a stream of purified nitrogen and the crystalline benzo(a)-pyrene was stored at -20°C until use the next day.

Previously, 190 M. inquinata and 100 kg sediment were collected from the low intertidal zone of Sequim Bay, Washington State, U.S.A., in an area of coarse sand mixed with fine gravel subject to moderate wave action. The sediment was passed through a 6 mm mesh sieve, and it and the clams were stored in the laboratory under flowing sea water at approximately 10°C and 30°/00 salinity. Detritus was collected from the laboratory seawater head tanks, filtered onto #42 Whatman filter paper, and refrigerated.

The labelled hydrocarbon compounds were individually dissolved in solvent as was a measured amount of Prudhoe Bay crude (PBC) oil. The solvent, oil, and labelled PAH were next mixed with detritus, and finally this slurry was incorporated into sieved sediment in a fiberglass-lined cement mixer, The final mixtures contained approximately 80 g detritus, 40 ppm PBC, and between 4.5 and 6.8 µCi labelled compound per kg. The final concentration of chrysene was 230 µg/kg, of phenanthrene 102 µg/kg, and of benzo(a)pyrene 42 µg/kg. Unlabeled phenanthrene present in the added PBC made up 23% of the total, while the amounts of chrysene and benzo(a)pyrene in the oil were negligible. These mixtures were poured to a depth of 8 cm into mesh-bottomed trays divided

into three compartments each. The trays were placed on cement blocks in fiberglass tanks at a depth which permitted 5 cm of water to stand above the sediment surface but which did not permit water to rise above the edge of the trays. At 12 hour intervals, water was pumped out of the fiberglass tanks for one hour, allowing the water in the trays to drain out through the mesh bottom and to be replaced by fresh volumes of water after the pumping stopped (Figure 1).

After two flushings, a sediment core was taken from each compartment and replaced by a 12 mm standpipe to facilitate future drainage. At this time, ten M. inquinata were placed in each compartment. The cores from the center compartment of each tray were divided into upper, middle, and lower sections. The radioactivity in replicate samples from each section was measured, and the results indicated that the labelled compounds were evenly distributed throughout the sediment. More than eighty percent of the calculated radioactivity added was recovered in the chrysene and benzo(a)pyrene exposures and 72% in the phenanthrene.

At intervals of one, three, seven, fifteen, thirty, and sixty days after the clams were placed in the trays, a sample of the surface water overlying the sediment was removed from one compartment and passed through a 0.45 μ Millipore filter. The water level was then lowered; ten clams were removed and placed in a mesh basket in clean running sea water for 24 hours deputation, after which they were rinsed with distilled water and frozen. Part of the sediment was also frozen immediately. The remaining sediment was placed in a Wildco CR™ Core Squeezer, in 100 cc batches, and the interstitial water was forced out with compressed air at 50 psi, passing through several layers of Whatman #42 filter paper within the squeezer. This filtrate was then passed through a syringe fitted with a Swinnex-47 Filter Holder (Millipore

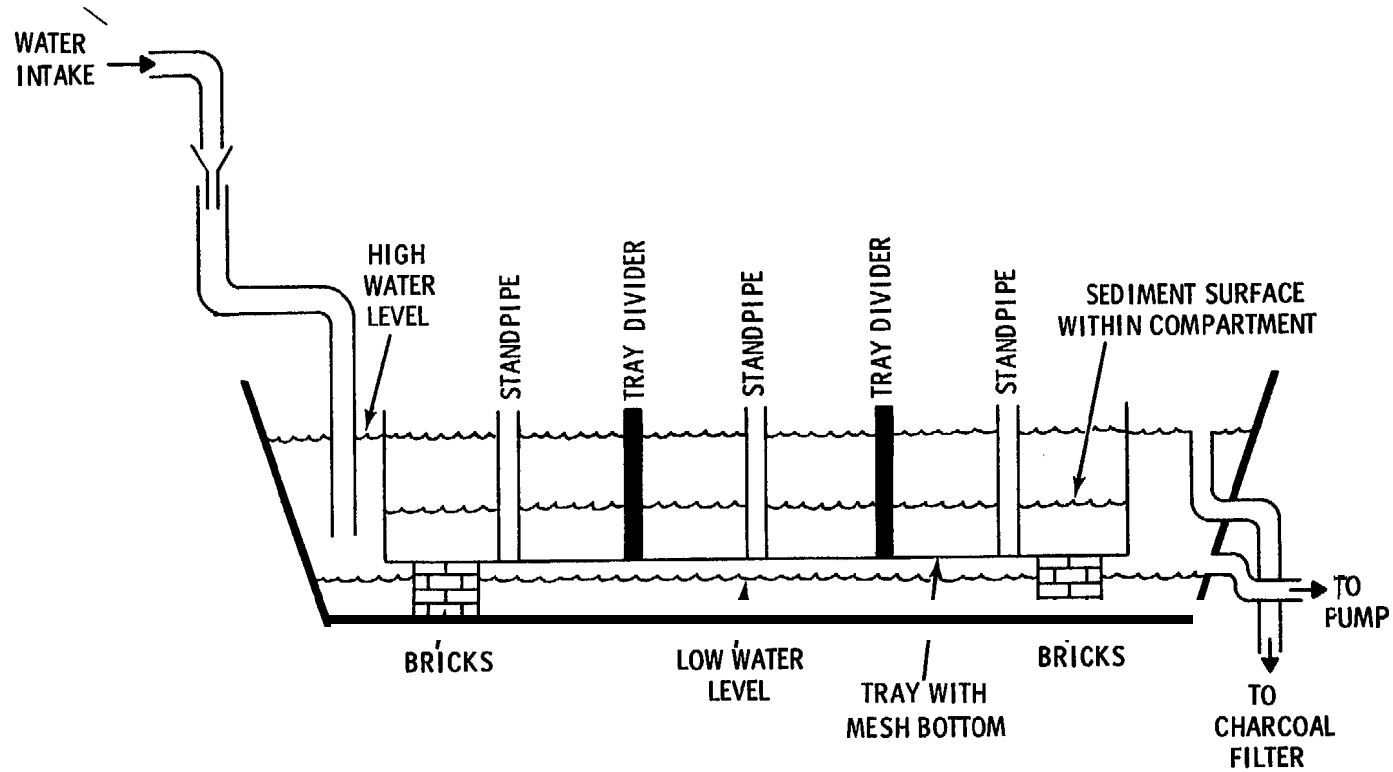

Figure 1. Design of sediment exposure system.

Corporation) containing a pre-filter and a 0.45 μ filter. A slightly different sampling schedule was followed in the benzo(a)pyrene exposure. Glassware containing benzo(a)pyrene in water was protected from light by aluminum foil wrappings. All water was kept in ice and protected from evaporation whenever possible.

Chemical Analyses

The total radioactivity present in the filtered interstitial water was measured by liquid scintillation spectrometry. Radioactive components contained in tissue and sediment samples were separated by reverse phase liquid chromatography and analyzed by liquid scintillation spectrometry. Stress was placed on an analytic approach which enabled us to account for the formation of degradation products other than CO_2 as a result of microbial activity or metabolism within the clams (Figure 2). Conventional tissue digestion techniques such as that described by Warner (1976) could not be used because of the potential chemical destruction of degradation products that might be formed during the experiments. Therefore, Macoma tissue samples were homogenized in 2:1 ethyl acetate/acetone. A similar extraction technique has previously been applied to a study of the metabolism of 7, 12-dimethylbenz(a)-anthracene in mouse skin homogenates (DiGiovanni et al., 1977), and it produced very high recoveries of original substrate and metabolizes. A modification of the techniques has been used in a study of benzo(a)pyrene phenols formed by the metabolism of benzo(a)pyrene by rat liver microsomes (Selkirk et al., 1974, 1976).

Tissue from individually shucked clams containing ^{14}C hydrocarbons was patted dry and weighed into 25 ml corex centrifuge tubes. To each tube was added a solution of ethyl acetate/acetone, 2:1 (3 ml/2.5 g tissue, wet weight).

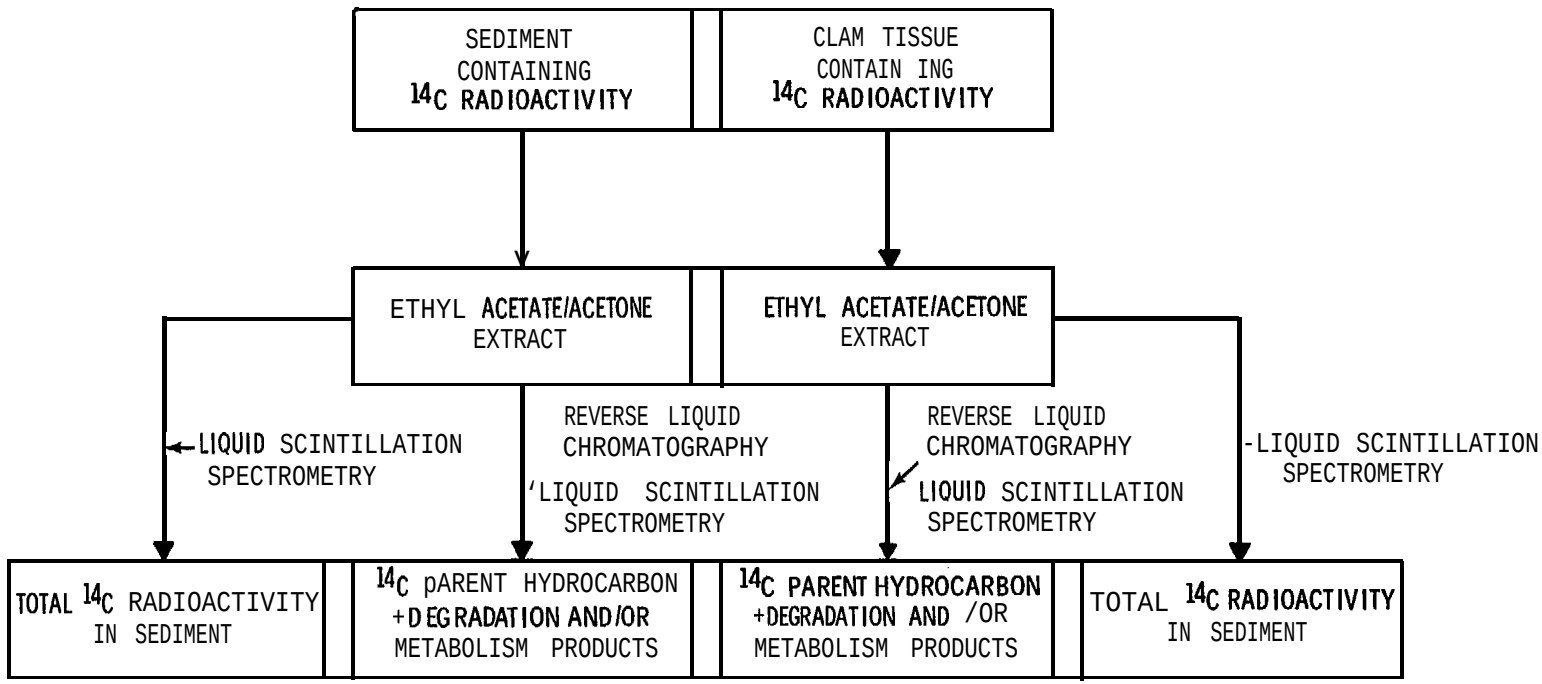

Figure 2. Scheme for the extraction and analysis of **^{14}C-labelled** hydrocarbons from clam tissue and sediment.

The samples were homogenized using a Tekmar Tissumizer and then centrifuged at 5000 rpm for 10 minutes. The organic layer (top) from each sample was transferred to calibrated 15 ml glass-stoppered centrifuge tubes with pasteur pipettes. The extraction sequence was repeated using ethyl acetate/acetone 2:1 (2 ml/2.5 g of tissue) and saturated sodium chloride (1.0 ml/2.5 g of tissue). The organic extracts (two from each tissue sample) were combined and the volume recorded. An aliquot of each sample was analyzed for total ^{14}C-radioactivity by liquid scintillation spectrometry. Recovery of ^{14}C-hydrocarbons from amended tissue was greater than 92%.

Samples of sediment from cores (~ 15 to 30 grams, wet weight) containing ^{14}C-radioactivity were Soxhlet extracted with 50 ml of 2:1 ethyl acetate/ acetone overnight. The Soxhlets were cooled in such a manner as to retain as much of the solvent as possible in the Soxhlet cup. The concentrated organic extract from each sample was transferred to calibrated glass-stoppered centrifuge tubes and the volume recorded. An aliquot of each sample was analyzed for total ^{14}C-radioactivity. A second overnight extraction of the sediment samples recovered no additional radioactivity in the solvent. Furthermore, no additional radioactivity was detected from direct liquid scintillation analysis of extracted sediment particles. These results indicated that use of this procedure resulted in high recovery of ^{14}C radioactivity from sediments.

One milliliter concentrates of tissue and sediment extracts were chromatographed on three series coupled μ-Styragel columns with pore sizes of 1000, 500, and 100 Angstoms and/or two series coupled μ Bondapak C-18 columns (reverse-phase). Methylene chloride was used as the mobile phase for the μ-Styragel system at a flow rate of 2.0 ml/min with the UV detector set at 254 nm. The same parameters were used for the reverse

phase system operating in the linear gradient solvent program mode (acetonitrile/water: 60/40 to 80/20 in 20 minutes). For both systems, aliquots of 2.0 ml fractions collected were analyzed for total ^{14}C-radioactivity.

RESULTS

Table 1 and Figures 3 to 5 show the changes in concentration with time of three PAH compounds in tissue and sediment. For convenience of comparison between compounds and between sediment and tissues all the hydrocarbon concentrations are given on a picomole/g wet weight basis. The data reflect the total amounts of hydrocarbon added to the sediment, including unlabeled compounds in the crude oil and radio-labelled material.

The fate of phenanthrene introduced into the sediment system differed from that of chrysene or benzo(a)pyrene (Figures 3-5 and Table 1). Fifteen days after the exposure began 50% of the phenanthrene initially present in the sediment had been removed, and after 60 days only 8% of the original amount remained. By contrast 67 and 77%, respectively, of the originally added chrysene and benzo(a)pyrene remained in the sediment after 60 days. Complementing its more rapid depletion from the sediment, phenanthrene or its metabolizes appeared in the interstitial water in concentrations two orders of magnitude higher than chrysene or benzo(a)-pyrene, so that after 60 days almost 3% of the activity remaining in the phenanthrene exposure system was found in the water.

The incorporation of radioactivity from phenanthrene into the tissues of Macoma also differed from that of the heavier compounds. The concentrations of chrysene and benzo(a)pyrene in the clams rose steadily over the 60 day period, while the sediment level fell. The tissue magnification factor

Table 1. Concentration of PAH in *Macoma* tissue and sediment ($\bar{x}$ ± S.D., n = 3), and the ratio between these two (magnification factor).

Days	*Macoma* (pmoles/g w.w.)	Sediment (pmoles/g w.w.)	Tissue/ Sediment	% Initial Sediment Content
		Phenanthrene		
0		396 ± 28		100
1	821 ± 234	407 ± 26	2.02	103
3	2486 ± 415	403 ± 84	6.17	102
7	2142 ± 940	364 ± 43	5.88	92
15	1608 ± 908	204 ± 84	7.88	51
30	574 ± 290	109 ± 59	5.27	27
59	103 ± 24	32 ± 5	3.22	8
59	*18 ± 4	*6 ± 1		
		Chrysene		
1	617 ± 83	750 ± 59	0.82	100
3	1399 ± 326	777 ± 159	1.80	104
7	1844 ± 754	735 ± 79	2.51	98
15	3697 ± 1697	773 ± 97	4.78	104
30	5013 ± 650	532 ± 79	9.42	71
58	5853 ± 656	503 ± 282	11.64	67
58	*1334 ± 149	*114 ± 76		
		Benzo(a)pyrene		
0		146 ± 5		100
1	79 ± 21	144 ± 1	0.55	99
3	167 ± 21	127 ± 110	1.31	87
11	262 ± 11	132 ± 19	1.98	91
20	429 ± 98	124 ± 3	3.46	85
35	453 ± 28	116 ± 28	3.91	80
60	588 ± 213	112 ± 11	5.25	77
60	*148 ± 54	*28 ± 3		

* ppb (wet weight).

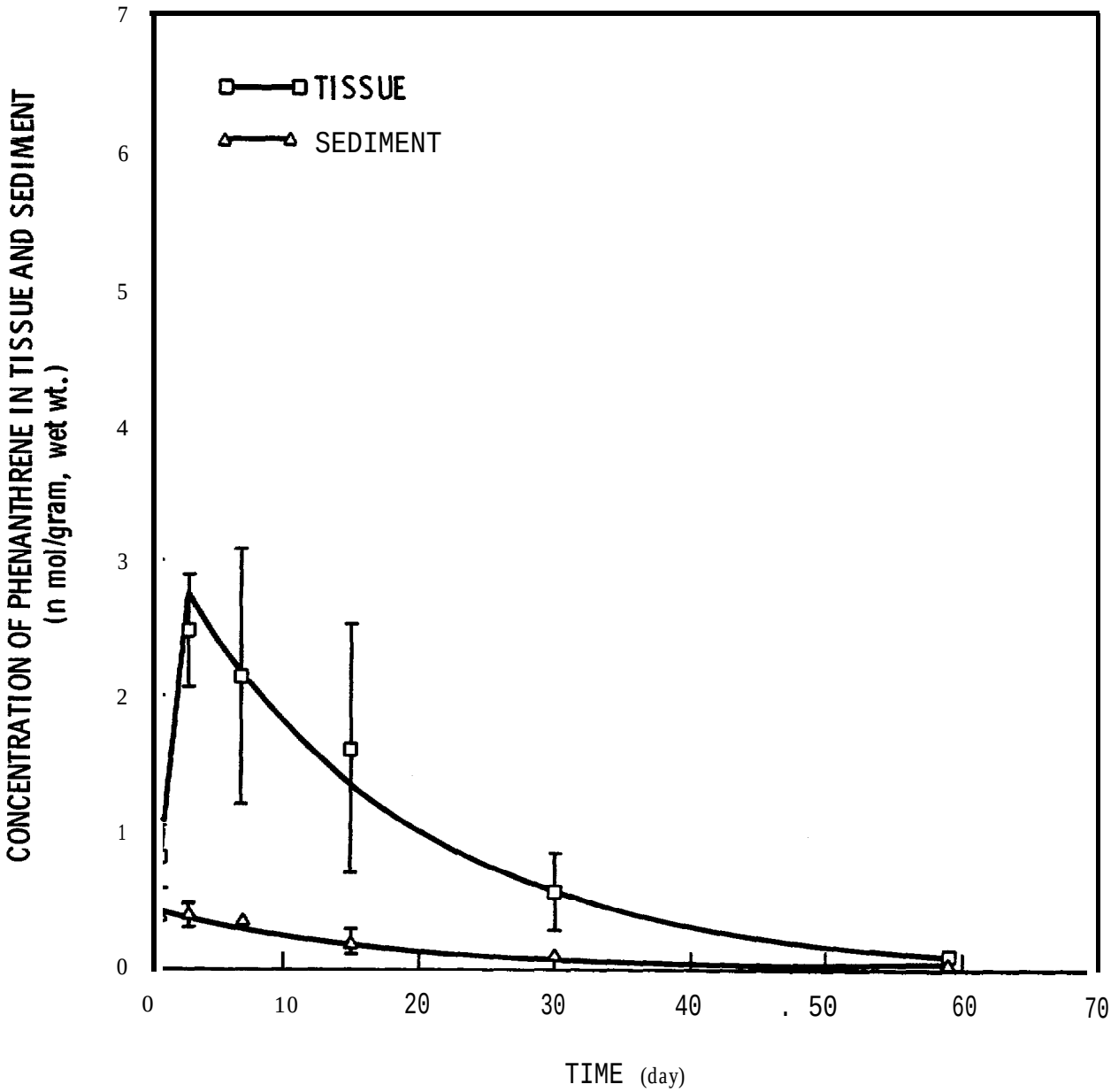

Figure 3. Concentration of phenanthrene in Macoma tissue and sediment (59 day exposure). The curves were fit by eye to connect the means (n = 3) and the vertical bars represent standard deviations.

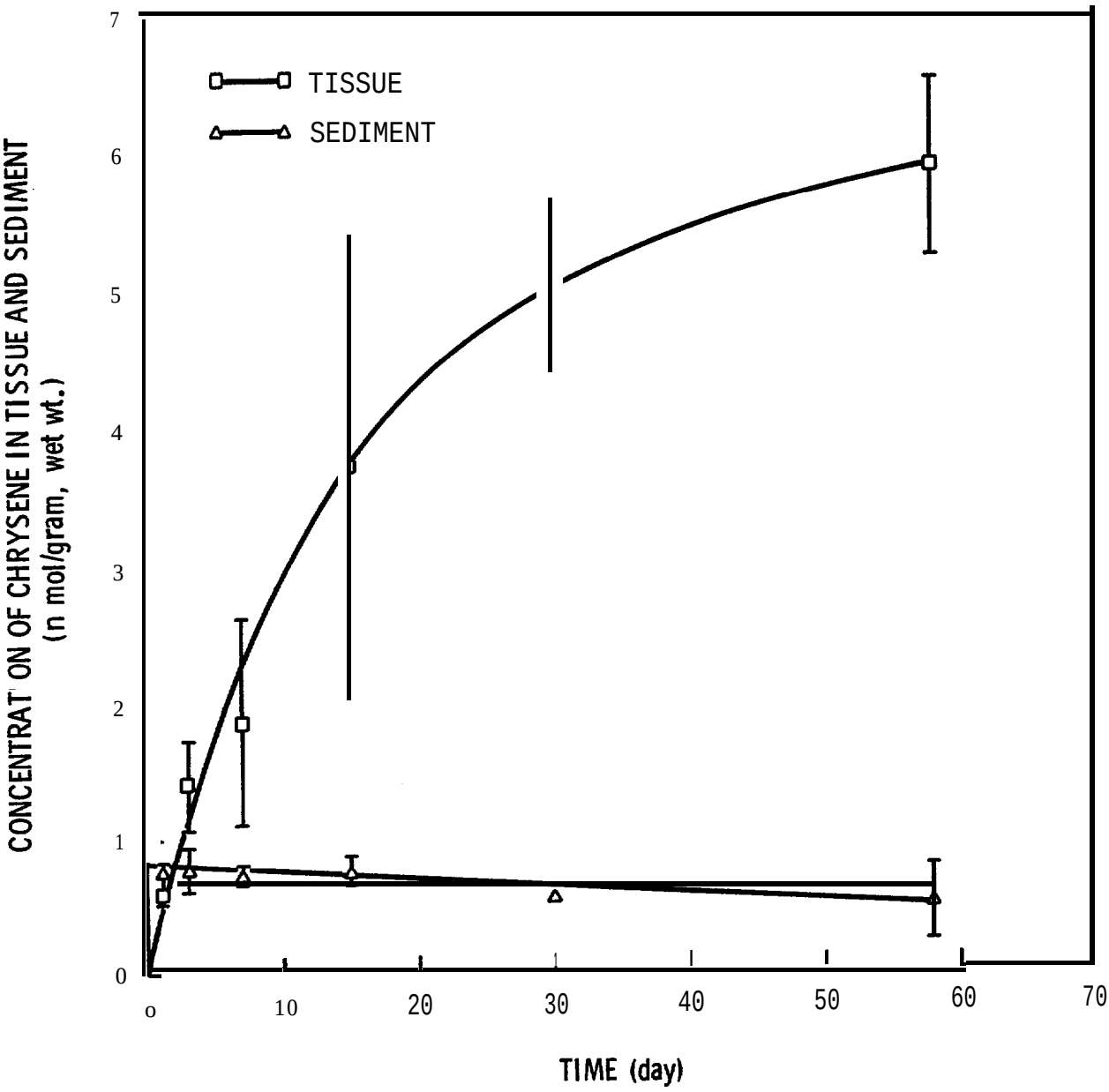

Figure 4. Concentration of **chrysene in Macoma tissue** and sediment (58 day exposure). The curves were **fit** by eye to connect the means (n = 3) and the vertical bars represent standard deviations.

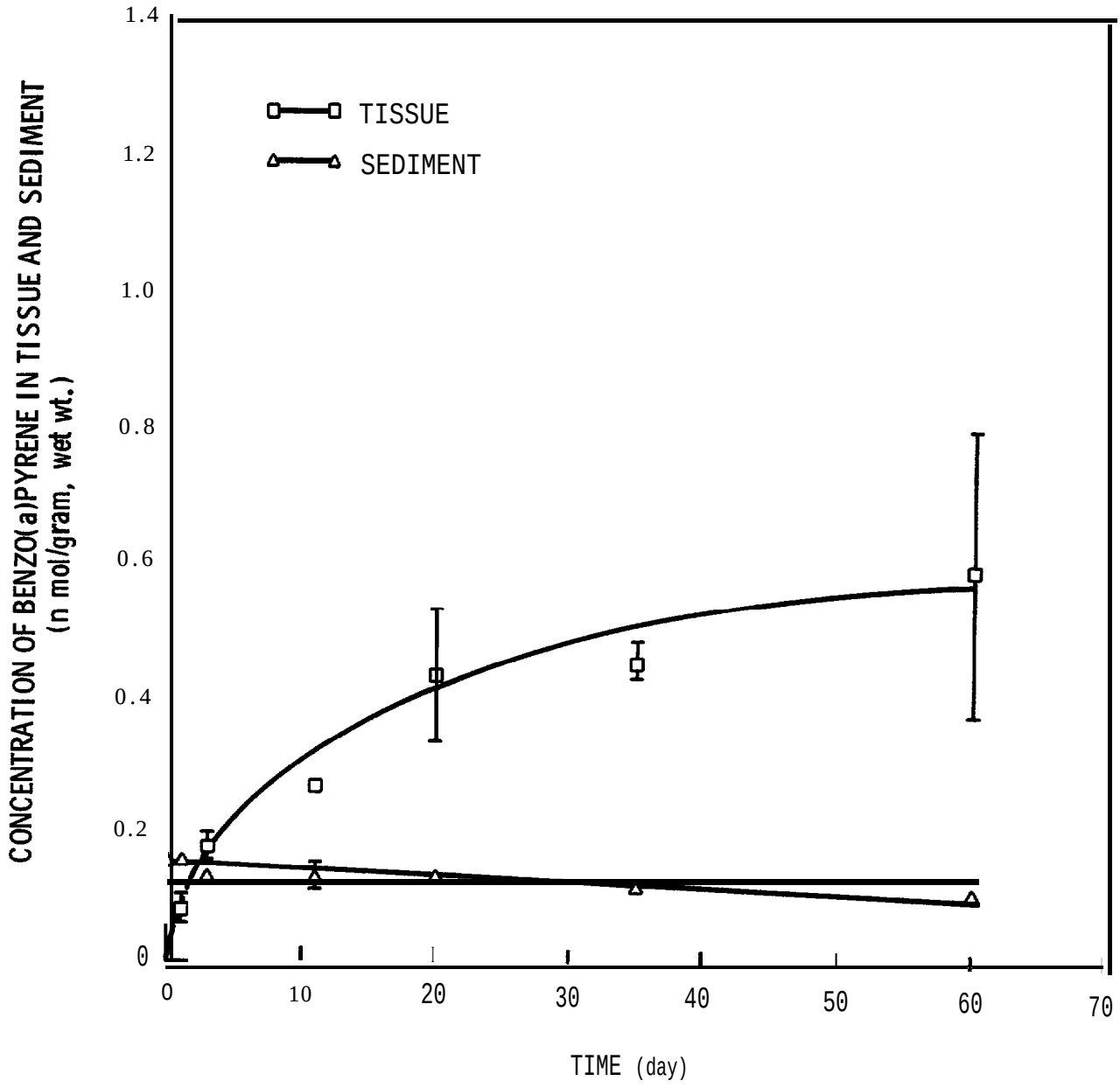

Figure 5. Concentration of benzo(a)pyrene in Macoma tissue and sediment (60 day exposure). The curves were fit by eye to connect the means (n = 3) and the vertical bars represent standard deviations.

therefore also increased continually reaching 11.6 for chrysene and 5.2 for benzo(a)pyrene, on a wet weight to wet weight basis (Table 1). Phenanthrene, on the other hand, was taken up in larger amounts than chrysene or benzo(a)-pyrene in the first three days of exposure, but its concentration fell thereafter. Since the sediment concentration of phenanthrene fell rapidly the tissue magnification factor continued to increase for two weeks, but it also declined towards the end of the exposure period. Put another way, the tissue content of chrysene and benzo(a)pyrene after 60 days exposure was 9.5 and 7.4 times as high as on day 1, respectively, while the tissue concentration of phenanthrene was only 1/8 as high on the last day of exposure as on the first (Table 1).

It is possible to roughly estimate the proportion of those hydrocarbon compounds leaving the sediment over 60 days which appeared in the clams at the end of this period by assuming that the total weight of the sediment in each compartment was approximately 6.6 kg and the total weight of the ten Macoma in each was 43 g. These are reasonable assumptions, based on the volumes of the compartments, the density of the sediment, and the average size of individuals of the populations of animals. Calculations based on these assumptions indicate that 15.4% and 11.3%, respectively, of the radioactivity of chrysene and benzo(a)pyrene which left the sediment can be accounted for by its presence in the clams, while only 0.18% of the corresponding phenanthrene remained in the organisms. Therefore, of the hydrocarbons present in the sediment when the clams were originally exposed, approximately 5.1% of the chrysene, 2.6% of the benzo(a)pyrene and 0.179% of the phenanthrene was present in their tissues after 60 days. Since these figures are not based on actual measurements of the animals' weight, they should be taken only as order of magnitude estimates.

No evidence was found for the presence of degradation products or metabolizes of any of the three compounds in either tissue or sediment. This conclusion is based on the findings that: (1) separation of tissue and sediment extracts by gel permeation chromatography (GPC) and by reverse phase high pressure liquid chromatography resulted in the generation, in each case, of one radioactive peak which had a retention time identical to that of the parent compound; and (2) recoveries of substrate radioactivity in tissue and sediment extracts from the GPC and reverse phase chromatography steps were high, indicating that no activity which could be ascribed to polar compounds had been lost on the column systems (Table 2).

DISCUSSION

Roesijadi et al. (1978) exposed M. inquinata to labelled phenanthrene, chrysene, and benzo(a)pyrene. Their system differed from the one described here in that the PAH hydrocarbons were distributed only through a thin (l-2 mm) layer of detritus overlying a clean sand substrate which contained the clams. Probably as a result of this arrangement a larger proportion of their added labelled material left the substrate, leaving less than one percent, five percent and twenty-five percent, respectively, of the originally added phenanthrene, chrysene, and benzo(a)pyrene at the end of the experiment. Consequently, in the earlier experiment the concentration of phenanthrene in the clams declined continuously from the start, and chrysene increased in concentration only for 15 days and then declined. Only benzo(a)pyrene followed the same pattern of continual increase in tissue concentration found here. This contrast in results illustrates the importance of the physical relation of the hydrocarbons to the sediment substrate for its bioavailability to the organisms.

Table 2. Percent recovery of ^{14}C-radioactivity from tissue and sediment extracts chromatographed on GPC and reverse phase liquid chromatographic systems.

Chromatography	Compound	Tissue[1]	Sediment[1]
GPC	Phenanthrene	101.5	97.6
	Chrysene	114.6	99.3
	Benzo(a)pyrene	102.1	102.6
Reverse-Phase	Phenanthrene	96.9	85.62
	Chrysene	103.8	101.4
	Benzo(a)pyrene	87.32	89.9[2]

[1] % recovery of activity injected on column system.

[2] Due to the low count levels in these samples, the lower recovery values can be accounted for by the greater counting error, which ranged from 10-30% for these samples.

The contrast, in this experiment, between the fate of phenanthrene and the fates of chrysene and benzo(a)pyrene may be accounted for by the tendency of hydrocarbons to remain sequestered in animals' lipids in inverse proportion to their volubility in water. Phenanthrene is more soluble, by three orders of magnitude, than the two heavier compounds (May, et al., 1978).

According to this model, half the added phenanthrene leaves the film surrounding the sediment particles within two weeks of exposure to dissolve in the water. The water-borne compound enters the clam tissue, producing the observed early peak of radioactivity. Once in the clam tissue, relatively more phenanthrene moves from lipids, entering the aqueous compartment which then exchanges with the surrounding water. At later time intervals the availability of phenanthrene in water probably decreases. A portion of this loss is probably due to microbial metabolism acting in the exposure system to alter the hydrocarbons in the interstitial water. Phenanthrene would be converted to soluble products, which would be eliminated from the exposure system by the daily flushing. As the sediment becomes depleted of phenanthrene the level of radioactivity in interstitial water falls, allowing the phenanthrene concentration in the clams to decrease by exchange with the relatively clean water.

Chrysene and benzo(a)pyrene, by contrast, remain within the oil film coating the grains of sediment for longer periods than phenanthrene. Though they enter the water phase more slowly, once they enter Macoma tissue they apparently remain longer in their lipid depots due to their slower rate of exchange with the ambient fluid. Such a sequence of events may be responsible for the time course of changes in tissue magnification factors seen in Table 1. The magnification for phenanthrene remains greater than that for chrysene for the first fifteen days of exposure, and greater than that for benzo(a)pyrene

for the first thirty. Nevertheless, the final tissue magnification factor is greater for the heavier compounds.

The absence of degradation products in tissue and sediments is consistent with the widely held belief that bivalves have very low aromatic hydrocarbon hydroxylase (AHH) activity (Payne, 1977). There is no doubt that bacteria in marine sediments are capable of metabolizing aromatic hydrocarbons. Furthermore, evidence of phenanthrene degradation was found in interstitial water from this experiment, and findings with regard to them will be discussed in a later publication. Probably any metabolizes formed were sufficiently soluble in water to readily leave the system during the flushing intervals.

The conditions chosen for these experiments are compared in Table 3 with those present in actual sediments and with those that might be expected after oil spills comparable in severity to the West Falmouth incident of 1969 and the Amoco Cadiz of 1978. In this table some values have been converted to a ppb dry weight basis for sediment and ppb wet or dry weight basis for tissues to facilitate comparison with data from other sources. Since the dry weight of the sediment was 84.3% of the wet weight there is little difference between these two bases. In the 1969 incident over 2000 ppm fuel oil was incorporated into sediment in some areas. If contamination of this degree with South Louisiana crude or Bunker C residual oil took place, the chrysene concentration in the sediment would be 34 or 400 ppb, (dry weight) respectively (Pancirov and Brown, 1975; taken from Neff, 1979). Giger and Blumer (1974) reported the presence of 40 and 240 ppb chrysene in sediment from relatively clean and chronically polluted sites in Buzzard's Bay, Massachusetts. The level of chrysene present at the beginning of this exposure, i.e., 203 ppb, is therefore, in an environmentally realistic range.

Table 3. PAH contents of sediment and tissues (ppb wet weight)

	Chrysene	Benzo(a)pyrene	Ref.
Sediment			
Laboratory, Initial	171 (203*)	37 (44*)	This study
Laboratory, Final	114 (136*)	28 (33*)	This study
Field, Buzzard's Bay, Massachusetts	240 (40*)	75-370*	Giger & Blumer, 1974
Field, Bay of St. Malo-Bassin		1 70*	Perdriau, 1964
Hypothetical contamination with 2000 ppm:			Pancirov & Brown, 1975
South Louisiana crude	34		
Kuwait crude	400	6	
Bunker C residual		88	
Tissue			
Laboratory, Final	1334	148	This study
(Concentration factor over sediment)	(11.6)	(5.2)	
Field, Oysters, Norfolk Harbor, VA	20-40		Cahnmann & Kuratsune, 1957
Field, Clams, Seine estuary		187	Perdriau, 1964
Hypothetical contamination of Macoma following 60 days exposure to sediment contaminated with 2000 ppm:			Pancirov & Brown, 1975
South Louisiana crude	265		
Kuwait crude		24	
Bunker C residual	3100	352	

* = ppb dry weight

The same is true for benzo(a)pyrene. Due to the large number of possible sources of this hydrocarbon, extreme values, as high as 15,000 ppb (dry weight) have been found in heavily polluted areas (Mallet et al., 1963). In a hypothetic sediment contamination with 2000 ppm Kuwait crude or Bunker C residual oil, 6 or 88 ppb (wet weight), respectively, of benzo(a)pyrene would be added to otherwise clean sediment (Pancirov and Brown, 1975). The laboratory exposure concentration of 44 ppb is within this range. The levels of 4- and 5-ring PAH accumulated over 60 days by the experimental animals is therefore probably of the same order of magnitude that might be expected in those deposit-feeding clams that survive the initial impact of a high-volume oil spill.

The tissue:sediment ratios of the specific aromatic hydrocarbons used in this study may be compared with the ratios of total aromatic hydrocarbons found in a field situation. Laseter et al. (1980) analyzed oysters and sediment taken from Aber Wrac'h, a site heavily polluted by oil from the Amoco Cadiz. Three months after the spill the total aromatic content of the Japanese oyster (Crassostrea gigas Thunberg 1793) was 1.24 times as high as that of the surrounding sediment. After fourteen months the ratio had increased to 2.30. The flat oyster, Ostrea edulis L., by contrast contained only 0.88 times as much aromatic HC as the sediment after three months, and after fourteen months the ratio had fallen to 0.53. It has been suggested that the difference between the species may reflect differences in the relative amounts of lipids they contain. The fact that the levels of bio-accumulation found here in Macoma were still higher than those in C. gigas, agree with the findings of Roesijadi et al. (1978) that deposit-feeding invertebrates tend to accumulate hydrocarbons at higher rates than suspension feeders.

Far more attention has been focused on the presence of benzo(a)pyrene in potential food organisms than on the presence of chrysene. This is understandable, since the former compound, when applied to mammalian skin, in very low doses (2 g/kg body weight) and in the presence of suitable co-carcinogens, can induce the growth of cancer. Though the lowest reported oral carcinogenic dose is 5 orders of magnitude higher, a degree of caution with regard to such a chemical may be warranted. It is possible, by extrapolating from the uptake observed under realistic conditions in the laboratory to the effects that might be anticipated in a post-spill situation such as those described above, to predict the presence of 25 to 350 ppb of benzo(a)pyrene in the tissues of deposit feeding clams. The upper part of this range is similar to that observed in molluscs taken from highly polluted areas such as the Bay of Naples (Boucart and Mallet, 1965) or the Seine estuary (Perdriau, 1964), or from creosoted pilings in Vancouver, B. C. (Dunn and Stich, 1975). The concentrations of benzo(a)-pyrene in smoked food or charcoal-broiled meat, two principal dietary sources, are an order of magnitude less than those in the more heavily contaminated bivalves (Lijinsky, 1967; Panalaks, 1976).

Fewer data are available on the range of concentrations of chrysene in tissues of marine animals, but Cahnmann and Kuratsune (1957) reported a concentration of 20-40 ppb in oysters from the Norfolk, VA., harbor. The potential for accumulation of this compound in bivalve tissue is therefore one to two orders of magnitude higher than that actually found in a fairly heavily polluted environment. The lowest reported carcinogenic dose of chrysene is four orders of magnitude higher than that of benzo(a)pyrene. Nevertheless its higher concentration in oil or oil products and its greater tendency to remain in tissues suggest that this compound may deserve some of the attention now being paid to the 5-ring PAH.

It has been demonstrated that a deposit feeding clam can accumulate heavy aromatic compounds to concentrations several times higher than those in surrounding oil-contaminated sediment. The findings imply that bivalves which survive the initial impact of oil contamination may become reservoirs for these compounds. Therefore, deposit feeding molluscs may be suitable choices for monitoring the long-term effects of oil pollution in sediments. Such monitoring would not only provide significant information on the state of contamination or deputation of an affected area, but also be useful in evaluating the degree of risk to predator organisms, including man, from ingestion of an additional dietary source of suspected carcinogens.

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II-B:

THE FATE OF POLYAROMATIC HYDROCARBONS IN AN INTERTIDAL SEDIMENT EXPOSURE SYSTEM: BIOAVAILABILITY TO ABARENICOLA PACIFICA (ANNELIDA: POLYCHAETA)

by

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ABSTRACT

Abarenicola pacifica, a burrowing polychaete, was exposed for 60 days to fine-grained sediment containing ^{14}C-labelled phenanthrene, chrysene, or benzo(a)pyrene. Thirty percent of the phenanthrene, but negligible amounts of the chrysene and benzo(a)pyrene present at the start of the exposure left the sediment. The concentrations of each of the aromatic hydrocarbons in Abarenicola tissue increased for two weeks to 4-6 times the sediment levels. The tissue concentration of chrysene remained constant thereafter, but the levels of phenanthrene and benzo(a)pyrene fell to one half of their peak values.

INTRODUCTION

An earlier paper in this series (Augenfeld, Riley, Thomas and Anderson, 1980) describes an exposure system and analytic scheme for investigating the fate and distribution of several ^{14}C-labelled polyaromatic hydrocarbons (PAH) in a moderately coarse sandy sediment. The uptake by, and fate of, these compounds in the intertidal detritivorous clam, Macoma inquinata, exposed to the sediment, was also examined. The work described in that paper has been extended to include studies of the fate of the same compounds i.e. , phenanthrene, chrysene, and benzo(a)pyrene in a more fine-grained silty sand sediment and their uptake by, and fate in, a non-selective deposit feeding polychaete, the lugworm, Abarenicola pacifica.

MATERIALS AND METHODS

Ninety-six A. pacifica and 100 kg sediment were collected from the high intertidal zone of an almost enclosed lagoon usually well protected from wave

action. The collecting site is separated by a narrow tongue of land from Sequim Bay, Washington State, U.S.A., from which the Macoma and sediment used in the earlier experiment had been taken. The grain size distribution of sediment from both sites was determined by the round hole sieve and hydrometer method.

^{14}C-labelled PAHs were purchased from Amersham-Searle Co., Arlington Heights, Ill. Benzo(a)pyrene was purified before use by the method described in Augenfeld, Riley, Thomas and Anderson (1980). Individual compounds were dissolved in small volumes of solvent together with Prudhoe Bay crude oil and spread over the surface of ca. 200 g sediment. After allowing about 20 minutes for the solvent to evaporate, the oil-PAH-sediment mixture was shaken by hand for two minutes and poured over a larger volume of sediment in a fiberglass lined cement mixer. The material was mixed for one hour and poured into 600-ml beakers whose bottoms had been replaced with 0.8 mm mesh Nitex. The sediment in the beakers contained 40 ppm PBC and a calculated activity of between 9 and 11 µCi labelled compound per kg. The calculated hydrocarbon concentrations were: 380 µg/kg of labelled chrysene, 169 µg/kg of labelled phenanthrene or 104 µg/kg of benzo(a)pyrene. The added oil contained negligible quantities of chrysene and benzo(a)pyrene but did include enough phenanthrene to make up 72% of the total phenanthrene in the sediment. In calculating the total concentration of phenanthrene in the sediment and tissues, the contribution of the unlabeled compound in the oil was taken into account.

The beakers were placed on racks in tanks in a sea table and flushed with running sea water for 18 hours. At this time 89% of the calculated amounts of labelled phenanthrene, 45% of the chrysene, and 79% of the benzo(a)pyrene were recovered in the sediment. Two specimens of A. pacifica were placed in 1/6 of the beakers, one each in 1/3 of the beakers, and none in the remaining 1/6. The water level in the tanks was continuously raised and lowered by a clockwork mechanism to produce a simulated "tide" which left the surface of the beakers uncovered for about 6 hours per day. This is similar to the tidal regimen which this population encounters in its natural habitat.

Beakers containing two worms were removed after one and three days, and those containing one worm after 7, 15, 30, and 60 days. Beakers containing only sediment were removed after 30 and 60 days. Sediment cores were taken from each beaker and frozen, and the remaining sediment was centrifuged for 30 minutes at 7500 g. The supernatant water was frozen for subsequent filtration through .45µ filters. The worms were rinsed and frozen immediately

after removal from the sediment. They were later thawed and rinsed with hexane, and their intestinal tracts were removed and slit open. The sediment within their guts was removed, the interior of the guts was rinsed with hexane, and the body wall and intestinal tracts were frozen together. When two worms were placed into one beaker, their tissues were combined for analysis.

The methods used for extraction and analysis of the tissue and sediment were the same as those described in Augenfeld, Riley, Thomas and Anderson (1980), with one modification. Due to the very fine grain size and high water content of the sediment, it was necessary to continue Soxhlet extraction for two days instead of one.

RESULTS

Table 1 shows the particle size distribution and water content of the sediments from the sites of origin of the Macoma and Abarenicola used in this and in the previous experiment. Approximately one half of the particles from both sites are fine sand, with grain size between 50 and 500 micra. In the Macoma collecting area 49.5% are medium and coarse sand and gravel with diameters greater than 500 micra. In the Abarenicola collecting area only 19.6% of the particles were in this range, while 28.2% were silt or clay with grain size less than 50 micra. The fine grained sediment contained twice as much water as the coarser material.

As Table 2 and Figures 1-3 indicate, the concentration of chrysene and benzo(a)pyrene in sediment changed little during the course of the experiments. The differences between the concentrations one day after and sixty days after the initial measurements are less than the variations among replicate samples. All the benzo(a)pyrene concentrations over this period are about 30% lower than that measured initially. This finding, however, may be due to an anomalously high initial measurement since the variation among replicates of the measurement was four times as great as that of any of the others. The weight of sediment in each beaker was about 1000 times as great as that of the worms they contained. It was therefore possible for the animals to accumulate substantial concentrations of PAH even though the concentrations in the sediment did not decline measurably. The sediment concentration of phenanthrene, by contrast to the two heavier compounds, did decline by about 30% over the course of the experiment. The sediment PAH concentration in beakers containing no worms did not differ significantly from those in beakers with worms.

Table 1. Particle size distribution and water content of sediments from **Macoma** and **Abarenicola** habitats.

Habitat	Water Content	Particle Size (μ)						
		Clay	Silt	Fine Sand	Medium Sand	Coarse Sand	Fine Gravel	Gravel
		<.002	.002-.05	.05-5	.5-1o	1.0-2.0	2.0-5.0	>5.0
Macoma	15.7%	1.1%	2.1	27.2	21.1	12.1	12.7	3.7
Abarenicola	29.4%	1.6	26.6	52.2	16.2	2.2	1.0	0.2

Table 2. Concentration of PAH in **Abarenicola** tissue and sediment **(pmoles/g ww)**

	Days	Abarenicola	(pmoles/g ww)	Sediment	% Initial Sediment Concentrations
Phenanthrene	0			968 ± 213	
	1	699 ± 100	.75	927 ± 95	90
	3	1253 ± 258	1.30	965 ± 0	100
	7	2553 ± 255	3.35	763 ± 44	79
	15	2940 ± 604	4.03	730 * 45	75
	30	1999 ± 629	3.13	639 ± 107	66
	60	1531 ± 167	2.27	674 ± 118	70
Chrysene	0			747 * 7	
	1	470 ± 173	.57	828 ± 27	111
	3	2097 ± 967	2.73	767 ± 105	103
	7	3821 ± 91	4.64	823 ± 31	110
	15	5014 ± 225	5.71	878 ± 68	118
	30	4401 ± 689	5.76	765 ± 52	102
	58	4490 ± 285	5.65	795 ± 98	106
Benzo(a)pyrene	0			328 ± 147	
	1	165 ± 40	.67	231 ± 7	70
	3	417 ± 65	1.86	224 ± 15	68
	7	912 ± 141	4.15	220* 14	67
	15	1197 ± 106	5.75	208 ± 36	63
	30	935 * 133	4.63	202 ± 22	62
	60	699 ± 88	2.85	245 ± 2	75

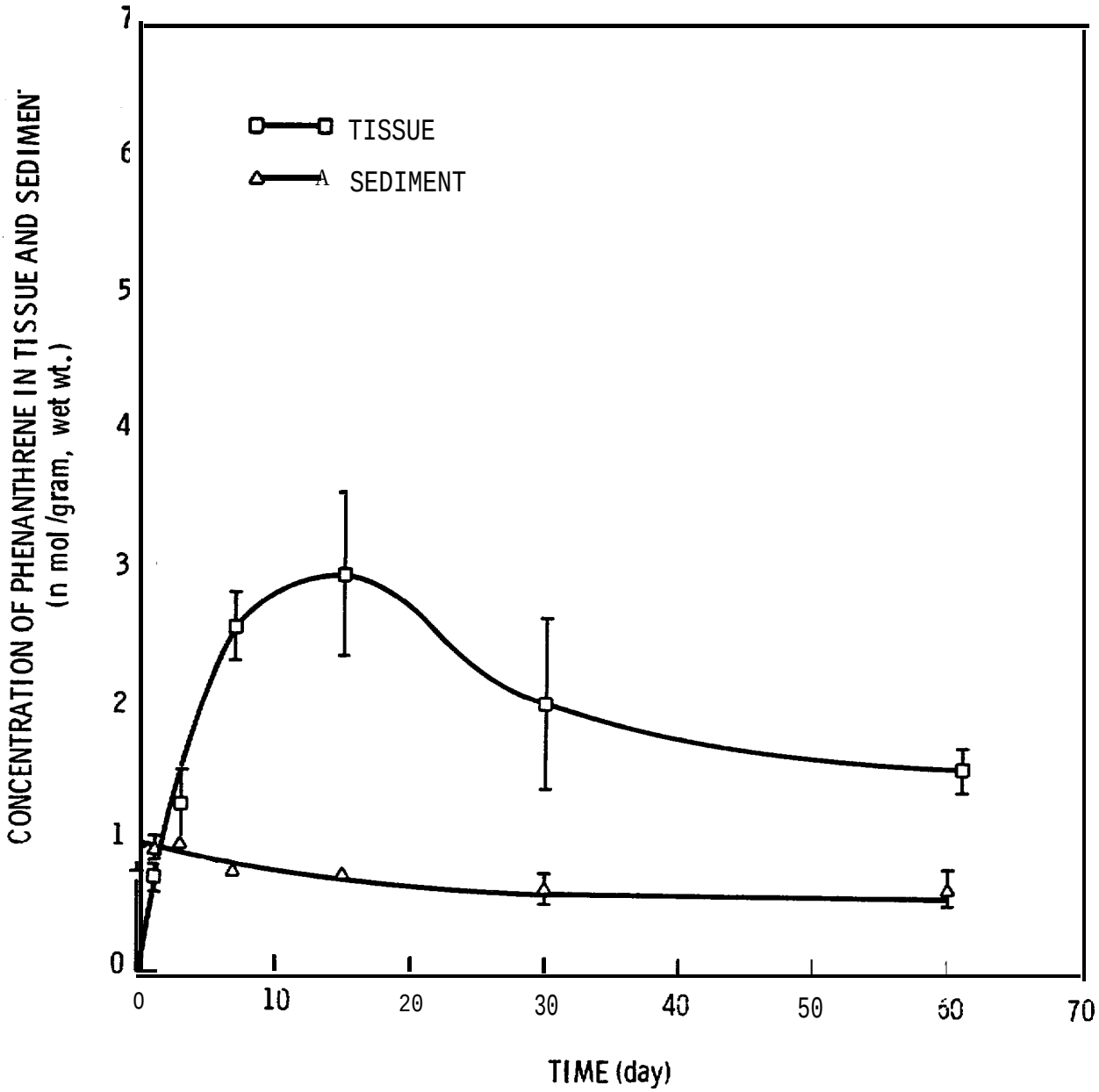

Figure 1. Concentration of phenanthrene in **Abarenicola** tissue and sediment (60-day exposure).

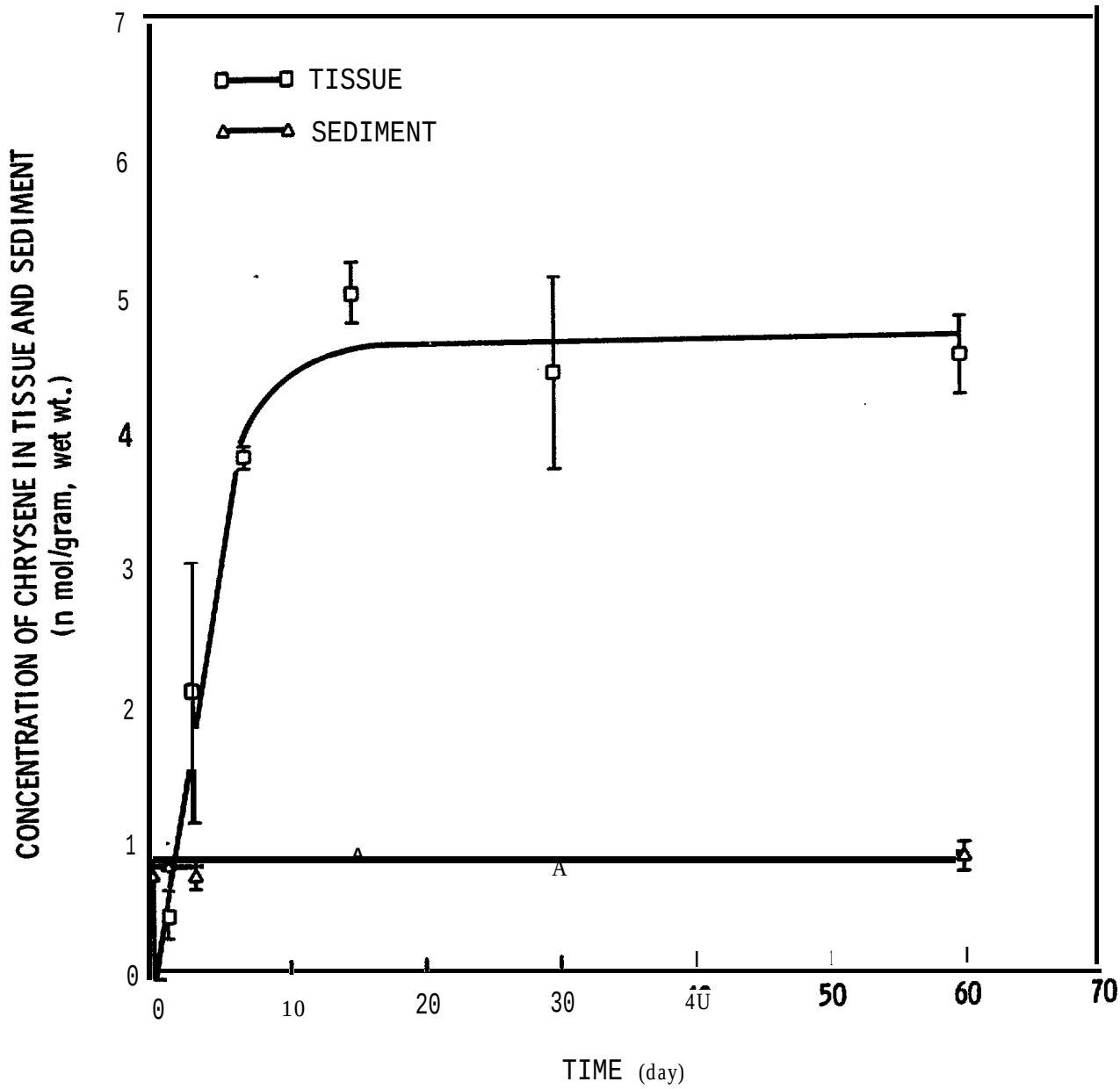

Figure 2. Concentration of **chrysene** in **Abarenicola** tissue and sediment (58-day exposure).

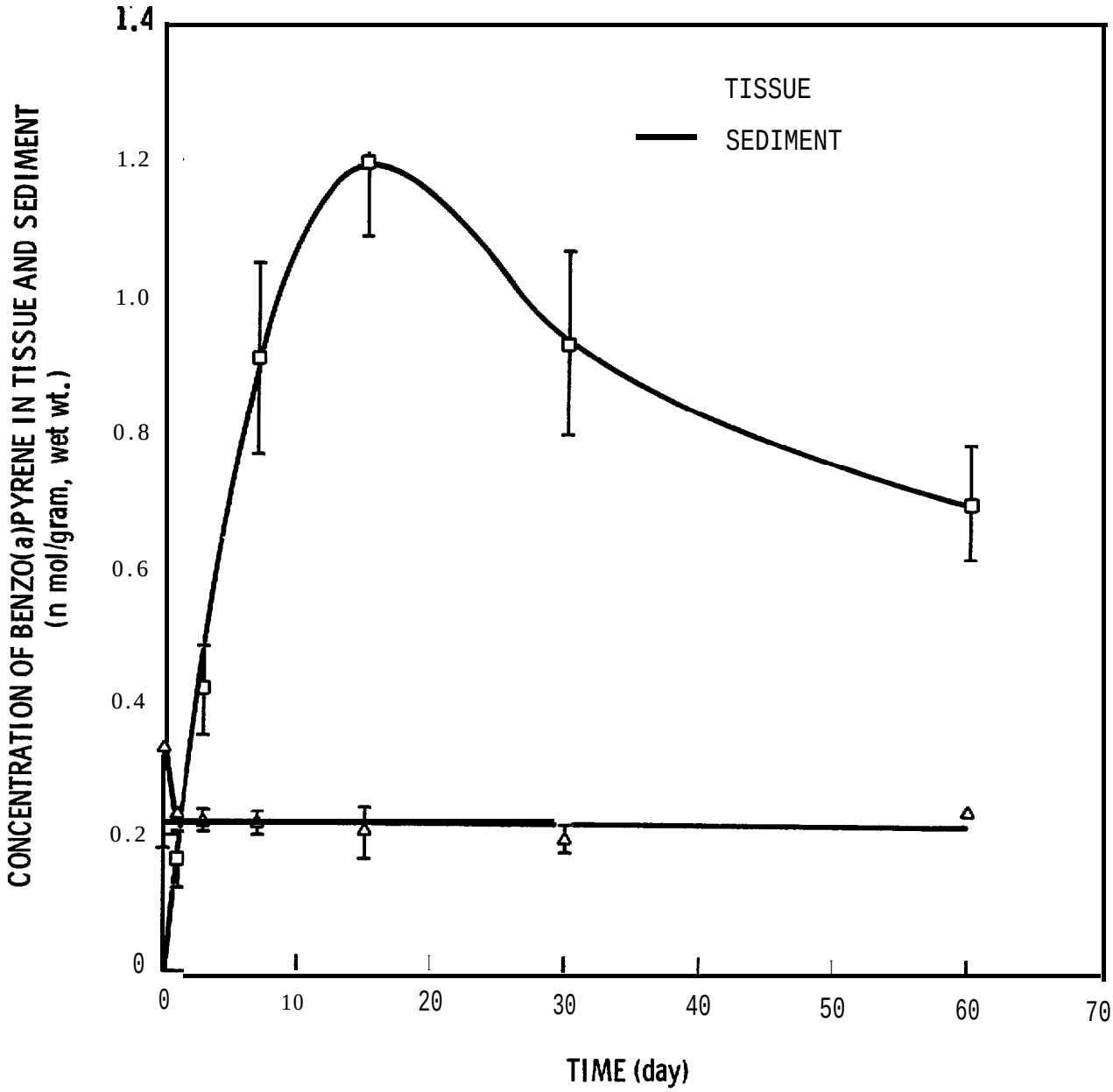

Figure 3. Concentration of benzo(a)pyrene in Abarenicola tissue and sediment (60-day exposure).

The concentrations of each of the PAHs in the worms' bodies reached a peak fifteen days after exposure began. In each case the tissue levels at this point were four to six times as high as the sediment levels at the time. In the case of phenanthrene and benzo(a)pyrene the tissue levels fell over the next 45 days to half of their peak values. After sixty days only two to three times as much PAH was found in the tissues as in the sediment. The tissue level of chrysene however decreased only slightly and the tissue magnification factor hardly changed.

No polar metabolizes of any of the three PAH compounds were found in either sediment or tissues.

DISCUSSION

In the coarser, dryer sediment employed in the study of Macoma referred to earlier, 92% of the added phenanthrene disappeared within 60 days, while less than two thirds of the added chrysene and benzo(a)pyrene did so. In the wetter, more fine-grained sediment occupied by Abarenicola all the compounds were held more tightly. The most striking contrast with the earlier findings lies in the fate of phenanthrene, 70% of which was still present after 60 days. Negligible amounts of the two heavier compounds were lost from this sediment. The considerably greater degree of retention is probably due to the lower rate of circulation of water through fine sediments.

Examination of the initial uptake of PAH by Abarenicola shows a steadily increasing concentration in the tissues, both in absolute terms and in proportion to the sediment level. Two weeks after the first contact however, the levels of phenanthrene and benzo(a)pyrene began to fall noticeably, even though sediment levels remained high. The reduction in tissue level following a lag period suggests the presence of an inducible enzyme system which could transform PAH into water soluble metabolizes that are more readily excreted.

Mixed function oxidases (MFO) such as aryl hydrocarbon hydroxylases (AHH) capable of converting benzo(a)pyrene into polar metabolizes have been found in several polychaetes, e.g., Nereis sp. (Payne, 1977; Lee et al., 1979) and Capitella capitata (Lee&Singer, 1979). Payne and May (1979) reported that they could find no detectable AHH in another lugworm, Arenicola marina, nor could they induce it by one week of exposure to ca. 3000 ppm Venezuelan crude oil. This finding is consistent with the fact that Abarenicola accumulated PAH in its tissue for two weeks before beginning to reduce their level,

since it indicates that an extended period of exposure may be required to stimulate AHH induction in lugworms.

The tissue level of chrysene remained essentially constant after the first fifteen days of exposure. This might indicate that, since chrysene is not as ubiquitous a compound as phenanthrene or benzo(a)pyrene, the MFO system of invertebrates has not developed as rapid a response to its presence. However, in Macoma chrysene also accumulated to a greater extent than either of the two other compounds. Clams are generally held to have no or very low levels of native or inducible AHH, and if this is the case, the higher levels of chrysene in Macoma tissue probably do not reflect a lower effectiveness in inducing enzyme formation. An alternate explanation for the higher level of retention in both species might lie in an affinity between the molecular structure of the compound and some component of invertebrate tissue.

If the effects of potential oil spills in areas inhabited by Abarenicola on the worms' bioaccumulation of PAH are to be compared with similar effects on Macoma, two factors which work in opposite directions must be balanced. On the one hand, the compounds are retained longer in the fine-grained sediment preferred by Abarenicola and are therefore more available for transfer to the tissues, by ingestion or absorption. On the other hand, the polychaetes are more capable of metabolizing and removing them from the tissues. Table 3 summarizes the point at which the balance is struck after 60 days exposure. It shows the ratio of labelled compounds found in the tissues of the two species to the amount of each compound present in the sediment on the first day of exposure. As can be seen, extended physical retention of phenanthrene in sediment outweighs the physiological ability of the worm to remove it during this time span. Since chrysene is retained longer in its surrounding sediment and is not excreted effectively, it too accumulates to a greater extent in Abarenicola than in Macoma. Only benzo(a)pyrene which is retained somewhat more in coarse-grained sediment, and is actively removed from Abarenicola tissue, is found in higher relative concentrations in Macoma

Two points however must be remembered in comparing the two species as depots of hydrocarbon pollutants. One is that the tissue levels found in Macoma may have been reduced during the 24 hour deputation period needed to purge their gut contents, while the Abarenicola were frozen immediately after removal from the sediment, and their gut contents rinsed out

Table 3. Ratio of tissue concentration of PAH after 60 days exposure to sediment concentration of PAH one day after contamination.

	Phenanthrene	Chrysene	Benzo(a)pyrene
Macoma	.25	5.44	4.02
Abarenicola	1.58	7.80	3.03

subsequently. The other point is that the tissue levels of phenanthrene and benzo(a)pyrene in the worms were declining at the 60 day interval, and a continuation of this trend for another 70 days would eventually reduce their body burden to sediment levels. In the clam, while phenanthrene was washed out of both tissue and surrounding sediment there was no indication that either chrysene or benzo(a)pyrene was released from the tissues. Therefore, if the results of exposure of invertebrates to PAH after actual oil spills resemble those found in this laboratory simulation, deposit-feeding bivalves such as Macoma could be expected to concentrate both chrysene and benzo(a)-pyrene to levels several times higher than those in the sediment, while polychaetes such as Abarenicola would retain only chrysene. The worms seem able to reduce their body burdens of phenanthrene and benzo(a)pyrene to bakcground levels within about four months.

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III. BIOAVAILABILITY OF METALS FROM PETROLEUM-IMPACTED SEDIMENTS

III-A:

BIOAVAILABILITY OF TRACE ELEMENTS FROM OIL-CONTAMINATED SEDIMENT TO MACOMA INQUINATA AND PHASCOLOSOMA AGASSIZII

Numerous trace elements including heavy metals occur in crude oil at low concentrations (Shah et al., 1970a, 1970b; Hitchon et al., 1975). Little is known about the dynamics of petroleum-derived heavy metals in the marine environment. Accumulation of specific heavy metals from sediment by benthic organisms has been described in numerous studies (Bryan and Hummerstone, 1971, 1973a, 1973b; Renfro, 1973; Hess et al., 1975; Luoma and Jenne, 1975; Renfro and Benayoun, 1975). The results, however, may not be directly applicable to petroleum-sediment-organism interactions since organic coatings on the surface of sediment particles can inhibit metals uptake (Luoma and Jenne, 1975). The presence of petroleum may interfere with the "normal" uptake kinetics of heavy metals from sediment. The low concentrations of trace metals in crude oil (e.g., 24 µg/g in Prudhoe Bay crude oil) suggest that the contribution of those metals to the environment will be very low. For example, sediment contaminated with 2,000 µg/g Prudhoe Bay crude will contain approximately 0.048 µg total trace metals contributed by the oil, assuming that all the metals in oil remain in the sediment. Compared to the normal levels of trace metals in marine sediments, that from oil would be negligible. Feder et al. (1976) were not able to detect any changes in sediment metals content as a result of contamination by Prudhoe Bay crude oil. However, experimental examination of the possible effects of oil on the uptake of trace metals has not been reported.

The uptake of trace metals from sediment contaminated with Prudhoe Bay crude oil has been investigated in two species of deposit-feeders: The sipunculid Phascolosoma agassizii and the clam Macoma inquinata. The animals were collected from intertidal regions of Sequim Bay and held at the Battelle Marine Research Laboratory under raw, flowing seawater at approximately 10°C and 30°/00.

In the first experiment individuals of Phascolosoma agassizii were exposed to oil-contaminated sediment (ca. 2000 µg/g) prepared by mixing sand and oil in a cement mixer. Exposures were conducted in sediment trays immersed in holding tanks containing flowing seawater. Animals were sampled after 1, 7, and 14 days of exposure and after 14 days deputation. Whole animals or tissue

homogenates were dried and analyzed by x-ray fluorescence. Secondly, Macoma inquinata were exposed for 2 weeks to oil-contaminated detritus. Fifteen g of oil-contaminated detritus (2000 µg/g) were supplied initially and on the seventh day of exposure. Clams were sampled at the end of exposure, allowed to depurate gut contents for 24 h, then dried. Tissue from 10 clams was pooled and analyzed by x-ray fluorescence.

A separate experiment was conducted to determine natural variability of trace metals in Macoma inquinata. Immediately following collection, 100 clams were placed in clean, flowing seawater for 5 days to allow deputation of gut contents. The clams were then dried and analyzed for trace metals by x-ray fluorescence. Ten samples of 9 to 10 clams each were analyzed.

Two samples of Prudhoe Bay crude oil were analyzed by neutron activation analysis. The concentrations of trace elements are presented in Table 1. Only four elements, vanadium, cobalt, zinc, and bromine, were present at levels above detection limits. The total concentration of trace elements was approximately 24 µg/g.

Several difficulties arose in the experiment designed to assess uptake of trace metals by Phascolosoma agassizii. Primary among these was the fact that the exposure sediment became anaerobic in the early stages of exposure, and exposed worms crawled onto the sediment surface. Therefore, worms may have been releasing to seawater rather than taking up metals from sediment during the exposure period. Such observations may account for the decreasing levels of compounds such as chromium, manganese, and nickel during exposure. Most were present at levels below those of the sediment, although a few such as nickel, copper, zinc, and manganese, were elevated compared to sediment concentrations. No discernible differences were observed between control and exposed groups. Values for aluminum, silicon, and titanium can be attributed to metals associated with sediment in the gut (Table 2).

Levels of trace metals in Macoma inquinata (Table 3) were generally similar to those of Phascolosoma agassizii, with a few exceptions: concentrations of chromium, manganese, iron, and nickel were substantially lower in M. inquinata despite the fact that the concentrations of the latter three metals in the respective sediment of the two species were quite similar. Nickel, zinc, and selenium were more concentrated in clam tissue than in sediment. Oil exposure did not affect metals concentrations in M. inquinata. With the exception of titanium and lead, individual variability

Table 1. Trace element concentrations in Prudhoe Bay crude oil. Samples represent oil from two different barrels and were analyzed by neutron activation analysis.

Element	Concentration (µg/g)	
	Sample 1	Sample 2
Na	<0.06	0.097
Mg	<3(.I	<33
Al	<0.5	<0.5
cl	<1	0.95
K	<4	<1.4
se	<0.001	<0.001
v	20.9	18.0
Cr	<0.21	<0.15
Mn	<0.04	<0.02
Fe	<1.6	<1.7
co	0.018	0.017
Cu	<5	<3
Zn	0.31	0.31
As	<0.03	<0.01
Se	--	<0.3
Br	5.73	2.75
Rb	<0.06	<0.08
In	<0.005	<0.003
Sb	<0.002	<0.002
Cs	<0.002	<0.001
Ba	<23	<8
La	<0.01	<0.01
Sm	<0.002	<0.001
Eu	<0.001	<0.001
Tb	<0.007	<0.006
Ta	<0.04	--
Hg	<0.03	<0.03
Th	<0.008	<0.006

Table 2. Uptake of trace elements by *Phascolosoma agassizii* exposed to oil-contaminated sediment.

Treatment	Concentration (µg/g dry weight)									
	Al	Si	P	S	Cl	K	Ca	Ti	V	Cr
Control										
sediment	60,500	35,500	-	-	-	8,200	29,400	4,550	33	690
sediment	60,700	37,200	-	-	-	8,500	30,100	4,810	61	703
animals from field	7,600	3,150	1,905	8,710	43,490	6,706	6,208	204	7.8	42
animals held in lab in sediment	5,070	2,716	1,606	9,953	38,650	7,600	2,344	242	6.0	29
animals held in lab 2 days out of sediment	5,800	1,310	395	10,500	49,700	6,820	3,480	153	<6	45
Exposed										
1 day - whole animals	5,000	437	1,536	11,300	43,400	7,350	1,850	100	<5	14
1 day - homogenate	3,400	1,300	790	9,490	32,034	6,230	2,326	145	<6	142
1 day - homogenate	2,000	1,224	1,680	9,?80	40,633	7,100	2,684	210	9.8	94
7 days - homogenate	19,450	1,584	2,408	11,140	46,190	9,174	3,986	128	6	47
14 days - homogenate	12,630	1,636	1,216	11, 150	49,320	9,478	2,323	83	<4	29
14 days + 14 days deputation - homogenate	20,990	1,673	<727	13,300	92,050	10,230	3,112	58	<4	15

	Mn	Fe	Ni	Cu	Zn	Se	Pb	As	Rr
Control									
sediment	443	17,900	<30	<2	<3	14	5.5	1.5	3.9
sediment	442	17,700	<30	<2	<3	12	2.0	0.8	3.5
animals from field	1,120	4,647	20	18	132	3.4	6.4	9.4	837
animals held in lab in sediment	4S2	4,256	24	9.2	109	8.0	5.9	12	751
animals held in lab 2days out of sediment	1,316	3,994	22	15	158	3.8	9.3	10	920
Exposed									
1 day - whole animals	180	2,824	19	20	154	3.1	5.5	10.5	822
I day - homogenate	1,087	3,673	49	21	251	4.5	15.5	13. t	?,026
1 day - homogenate	1,385	3,947	44	19	185	4.2	19	12	814
7 days - homogenate	598	3,494	31	12	145	<.3	9	12	712
14 days - homogenate	273	2,629	13	12	80	<.5	<1	12	696
14 days + 14 days depuration - homogenate	123	2,241	9	13	112	<.5	<1	11	810

Table 3. Uptake of trace elements by *Macoma inquinata* exposed to oil-contaminated detritus (ca. 2000 µg/g total hydrocarbons).

Treatment	Concentration (µg/g dry weight)						
	Cl	K	Ca	Ti	V	Cr	Mn
Control							
Initial seal.	16,800	11,900	21,100	4,690	80	145	668
2-wk seal.	16,400	11,600	20,900	4,610	109	101	706
Initial tissue	52,900	13,000	1,970	21	<4	<2	11
2-wk tissue	55,200	13,900	1,590	29	<4	2.7	12
2-wk tissue	51,100	12,500	2,030	55	❑4	5.6	19
Exposed							
Initial seal.	15,400	11,100	19,600	4,460	84	126	682
2-wk seal.	16,600	11,400	20,400	4,500	73	97	706
2-wk tissue	55,500	13,200	1,870	42	<4	5.5	14

	Fe	Ni	Cu	Zn	Se	Pb	As
Control							
Initial seal.	38,800	<36	31	85	1.3	10	9.2
2-wk seal.	38,600	<32	34	92	1.4	12	13.1
Initial tissue	284	4.2	7.7	199	3.1	<1.2	11
2-wk tissue	370	3.5	9.1	210	3.1	<1.3	11
2-wk tissue	516	4.9	10.3	202	2.7	<1.3	11
Exposed							
Initial seal.	37,400	<43	31	84	1.5	10	10.1
2-wk seal.	38,100	<33	35	90	1.3	13	10.1
2-wk tissue	535	3.3	7.5	163	2.9	2.1	8.2

was relatively low for all metals (Table 4) (combined coefficient of variation = 12.3 ± 5.5 S.D.). Titanium was most likely to be associated with sediment remaining in the gut and would therefore be more likely to exhibit a degree of inconsistency. Concentrations of lead approached detection limits of the technique. Only three samples possessed measurable quantities of lead. Only the values for chromium and manganese in the previous experiment fell outside the 95% confidence limits determined in this study.

Since it is possible that x-ray fluorescence techniques may not detect small changes in the tissue content of certain heavy metals, the use of radio-labelled detritus and oil, which would be produced by neutron-activation of these substances, was suggested. By generating gamma-emitting isotopes from the metals contained in the oil and associated with the detritus, very small amounts of isotopes transferred from these substances to the detritivores could be measured. In the fall of 1977 samples of oil and detritus were subjected to neutron-activation, and the products were measured for isotope content and activity. Because the concentration of metals in the oil was so low (Table 1) and the specific metals present did not lend themselves to use in this experimentation, the activated oil was not utilized.

The detritus, however, did possess at least four gamma-emitting isotopes which exhibited activities and half-lives suitable for use in experimentation. In January of 1978, a preliminary experiment was conducted to evaluate the uptake of isotonically label led heavy metals by the clam Macoma inquinata. Activated natural detritus was mixed with fresh cold detritus (1:10) and the mixture was "aged" in seawater at 10°C for four days. The final product was then filtered on #42 Whatman paper, and divided into two halves. The oil-impacted portion received a calculated 2000 ppm of PBC contamination by the methods described earlier under hydrocarbon exposure. The non-oiled portion received only one ml of ether used as a carrier in the oiled sample. These two samples of activated detritus were placed on the bottom of two separate 5-liter aquaria, and low aeration was supplied. Ten marked clams were placed on the substrate of each tank, and then a basket containing an additional five clams was suspended in the water column above the other animals. The exposure continued for one week, followed by a deputation period of two days. During the exposure, water and clam samples were

Table 4. Analysis of trace elements in *Macoma inquinata* by x-ray fluorescence. Estimation of sample variability.

Element	Sample Size	Concentration (µg/g) x ± 2 S.E.
P	10	4,651 ± 686
s	10	15,374 ± 59?
cl	10	53,859 ± 3,695
K	10	13,504 ± 245
Ca	10	2,003 ± 140
Ti	10	23.7 ± 9.5
v	3	3.58 ± 0.45
Cr	5	3.92 ± 0.60
Mn	10	9.136 ± 1.043
Fe	10	315.2 ± 31.3
co	4	2.497 ± 0.442
Ni	10	3.282 ± 0.391
Cu	10	8.108 ± 0.374
Zn	10	195.2 ± 12.5
Ga	10	n.d.[2]
Hg	10	n.d.
Se	10	3.177 ± 0.188
Pb	3	0.815 ± 0.680
As	10	10.319 ± 0.368
Br	10	262.5 ± 17.8
Rb	10	n.d.
Sr	10	29.59 ± 2.46

[1] In sample size <10, the remaining samples (=10-n) were below detection limits. Nine to ten clams composed a single sample.

[2] n.d. = not detectable.

counted at 1, 3, and 7 days, and animals were also counted after deputation. It was possible to utilize a small number of animals since they could be counted alive and placed back in the aquarium. The same groups of five individuals were counted together, and the configuration within the counting chamber was kept constant. After the one-week exposure and final counting at Sequim for total gamma activity, five of the ten animals on the bottom of each aquarium were transferred to clean water with clean detritus for two days deputation. The remaining five in each group were removed from the shell, and both tissue and shell were analyzed in detail at the Richland laboratory of Battelle, Pacific Northwest Laboratories. The same procedure was used on the 2-day depurated groups and the two groups of five suspended above the detritus. The determinations of total gamma activity and specific isotope content of the various groups and samples are shown in Tables 5 and 6.

During the one-week exposure, the oiled detritus decreased in total hydrocarbon concentration from 1755 ppm to 1138 ppm. The gamma activity associated with the water above the detritus (both oiled and non-oiled) was primarily in solution and was of significant magnitude, except on day 3 (Table 5). Counts generally present in the 200-ml water samples were about twice as high as those found in the clam tissues after seven days of exposure to detritus (200). Clams suspended above the substrate, where activity could only be obtained from the water and very fine suspended particles, exhibited rather consistent counts between 34 and 79. The shells of clams living on the bottom of both aquaria (oiled and unoiled detritus) had a total activity of 35 counts/g (per 40 rein). Two days of deputation in clean water and detritus reduced all counts, including those clams on and above detritus, with and without oil and shells, to a range of 23 to 52 (Table 5). Since the shell alone gave a count of 28, it is apparent that uptake by clam tissue was extremely small, if present at all.

Samples taken on the seventh day of exposure and after two days of deputation were analyzed for content of specific radioisotopes (Table 6.) It is clear that the detritus contained sufficiently high amounts of these four isotopes to provide the organisms with an opportunity to exhibit uptake. More ^{60}Co than other metals was found in the water, but it amounted to only 1% of the detritus activity. The Zn in water equalled about 4% of its detrital concentration. There are no apparent differences

in the metal contents of clams between the oiled and non-oiled groups, but the sub-groups living above the detritus both exhibited lower contents. Deputation for two days reduced the levels to those of the clams living above the substrate, which were approximately equal to those associated with the shells of those living on the detritus.

It is interesting to note that when the Zn counts in the tissues are converted by use of the Zn specific activity, the amount of Zn accumulated by Macoma represents only about 0.1% of the total Zn found in freshly collected animals. These findings make two facts apparent. First, no other means of analysis would ever detect uptake of Zn at this very low level; and secondly, a short deputation reduces tissue levels to approximately the same activity associated with shell material.

A final experiment was planned using detritus containing a larger number of isotopes and higher specific activity. The results of this study are described in the next section of this report.

Table 5. Uptake of total gamma-labelled trace metals from detritus by Macoma
Values are counts per 40 minutes per gram (tissue) or per 200 ml (seawater).

Type of Sample	Sample Interval (days) Exposure				Deputation (after 7 days exposure)
	0	1	3	7	2
Seawater (200 ml)					
Filtered (0.5μ)	370	432	23	440	
Unfiltered	473				
Filtered	268				
Macoma					
On detritus					
with oil		109	141	200	45
without oil		230	59	172	52
Above detritus					
with oil		79	54	68	27
without oil		68	34	55	23
Shell only				3	5 28

Table 6. Uptake of specific radio-labelled trace metals from detritus by *Macoma*. Values are counts per 1000 minutes per gram detritus or tissue and per 200 ml seawater.

	Isotopes			
	^{152}Eu	^{60}Co	^{46}Sc	^{65}Zn
Detritus (after 7 days)				
With oil	25,984	13.456	14,528	1,216
Without oil	17,038	17.038	9,656	913
Seawater (200 ml)				
Filtered on day 7				
With oil	0	131	1	41
Without oil	14	173	7	28
Filter				
With oil	6	1	9	7
Without oil	8	6	5	<1
7-day *Macoma*				
With oil				
On detritus	101	70	55	16
Above detritus	10	13	4	7
Without oil				
On detritus	81	65	48	13
Above detritus	7	11	4	8
2-day deputation (On detritus)				
With oil	15	19	8	7
Without oil	23	26	10	13
Shell only				
7-day exposed on detritus				
With oil	39	24	22	7
Without oil	50	28	23	8
7-day exposed above detritus				
With oil	16	9	8	5
Without oil	13	9	7	4
2-day depurated (On detritus)				
With oil	37	22	20	7
Without oil	10	10	10	4

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III-B:

UPTAKE OF TRACE METALS BY THE CLAM *MACOMA INQUINATA* FROM CLEAN AND OIL-CONTAMINATED DETRITUS

by

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In recent years there has been increasing concern about the entry of petroleum hydrocarbon (PHC) into the marine environment and the effects of such entry on the composition and functioning of the marine ecosystem. Few reports have been published on the possible effect of oil on the uptake of metals from water or sediments by animals. The possibility of such effects is indicated by the work of Fletcher *et al.* (1979), who showed that crude oil causes a reduction in blood plasma copper concentrations in fish, and Payne *et al.* (1S78) who reported that petroleum affected chloride regulation in fish. Luoma and Jenne (1977) have shown that the availability of sediment-bound metals to a deposit-feeding clam depended on the metal-sediment association and sediment-to-water resorption rate.

Here, we exposed a detritivorous clam, *Macoma inquinata*, to clean and oil-contaminated detritus to determine the effects of the oil on metal accumulation. To measure the uptake of metals, clams were exposed to neutron activated detritus and the uptake of several isotopes (^{51}Cr, ^{60}Co, ^{152}Eu, ^{59}Fe, ^{46}Sc, and ^{65}Zn) measured in the clams.

Preparation of detritus

The term "detritus" as used in this paper is defined as the suspended matter in Sequim Bay water that settled out in the head tanks of the laboratory flowing seawater system. The element composition of this detritus is similar to both that of Sequim Bay fine grained sediment and shale (Vinogradov, 1962).

Detritus was collected from the bottom of head tanks through which raw Sequim Bay seawater had passed. Dried detritus was neutron activated for two hours at a neutron flux of approximately 1 x 10^{13} n cm $^{-2}$ sec $^{-1}$, and stored for three months to allow activity to be reduced through radioactive decay. Six g dry weight (d.w.) of neutron activated detritus was added to 100 g wet weight of untreated detritus in 1 L of sea water, and the mixture was shaken by hand for 1 minute. The resulting slurry was aerated for five days at 13*C, then filtered onto #42 Whatman paper and the detritus divided into two portions of approximately 60g each. Enough Prudhoe Bay crude oil to produce a concentration of 1000 ppm oil in 609 of the detritus was dissolved in 1 ml of ether. The ether-oil mixture was then added to 100 ml sea water containing 60g of detritus. To the second 60 g portion of detritus was added 100 ml seawater and 1 ml ether without oil. Each portion of detritus was shaken 4 minutes, filtered again, and samples of the detritus removed for gamma counting.

Exposure system

The exposure system was designed to expose clams to either oiled or non-oiled detritus, and also expose other clams to only water-borne materials defined as less than 100 µm in diameter. The exposure system consisted of

two 4 L aquaria that had been divided into two equal compartments. Oiled or non-oiled detritus was placed in one compartment of each aquaria and 2 L seawater added to each compartment. Water was pumped from the compartment containing detritus through a 100 µm mesh nylon screen into the adjacent compartment. Water returned to the detritus containing compartment by gravity flow over a separating barrier. The clams were dug and stored in a flowing aquarium without food, for two days before the experiment began. Thirty clams were placed in each of the four compartments. To maintain a constant temperature the aquaria were placed in a 13° water bath.

This system provided for the exposure of clams to detritus and water-borne materials (<100µm) on one side and only water-borne materials (<100µm) on the other. The pump system provided the necessary aeration without agitating the detritus enough to suspend it in the water column. There was a small amount of solids that accumulated on the bottom in the filtered compartment. These were removed periodically by gentle suction and returned to the compartments with detritus.

Groups of five clams were removed from each compartment after 2, 4, 8, and 15 days of exposure. They were opened, and the meat and shells were rinsed in fresh sea water. If needed, shells were scrubbed before being opened to remove adhering detritus. The meat and shells of each group were dried to constant weight at 80°C. One-hundred ml samples of sea water were taken from the filtered compartment after 2, 8, and 15 days and evaporated to dryness for counting. Samples of oiled and non-oiled detritus were removed and dried on the 15th day of exposure, for gamma counting.

After 15 days of exposure, the remaining clams were transferred to deputation tanks with clean water and non-labelled detritus, both of which were replaced after 2 and 4 days. Groups of five clams were removed

after 2 and 8 days of deputation and prepared for counting. All meat and shells from 1 group were pooled. The gamma activity of samples of detritus, meat, shells, and residue from evaporated sea water was measured on a Ge(Li) diode.

Data treatment

The numbers of net counts at energy levels corresponding to ^{51}Cr, ^{152}Eu, ^{46}Sc, ^{59}Fe, ^{65}Zn, and ^{60}Co were calculated and corrected for the rates of decay of each isotope to determine the count rate/g d.w./1000 minutes at the time each sample was removed from the experiment. The relation between the corrected isotope count and the actual amount of metal present was established by reference to the known metal content of the detritus. These values had been established in this laboratory for K, Ca, Ti, V, Cr, Mn, Fe, Cu, Zn, Se, Pb, and As, by x-ray fluorescence. The elemental values we measured agreed with the trace metal concentrations in shale as reported by Krauskopf (1967); taken from Vinogradov (1962). Since the elemental composition of the collected detritus was similar to that of shale, we used the levels of Eu, Sc, and Co in shale in calculations to determine the amount of metal originating from detritus that was present per g clam meat or shell, or per ml sea water.

Since the clams that were removed from the aquaria during the exposure had not been depurated to purge their intestinal tracts before they were shucked and dried, it was necessary to partition the total Isotope content of each metal in meat samples into that portion which was in transit through the animals' digestive systems at the time of sampling and that which had passed through the gut wall and been incorporated into the tissues. This partitioning was made possible by the presence of ^{46}Sc and ^{152}Eu in the

detritus. Based on work by Palumbo (1963) and Peters and Hess (1974) there is good reason to believe that these two elements, as a result of their chemical form and insolubility, are absorbed very poorly if at all from food into animal tissue. The amount of detritus localized in the gut lumen was, therefore, taken to be equal to the amount of shale which would contain the scandium present in the entire sample. The amounts of other metals localized in the gut were calculated by multiplying the concentration of each metal in shale by the calculated quantity of detritus in the gut. To determine the amount of each labelled metal in tissue, the amount present in the gut was subtracted from the total amount present in the sample.

Orte example of this treatment of the data is given here:

Metal	Concentration in shale	Concentration in detritus	Corrected Counts /1000 reins/g d.w. detritus	Corrected Counts /1000 reins/ g d.w. Macoma (non-oiled, 4 days)
Sc	10µg/g		287,907	4086
Zn	80µg/g	88µg/g	3,493	90

$\frac{3493}{88} = \frac{90}{x}$ x = 2.27 µg Zn/g d.w. total Macoma sample.

$\frac{287,907}{10} = \frac{4086}{y}$ Y = .142 µg Se/g d.w. total Macoma sample.

$\frac{10}{10^6} = \frac{.142}{z}$ z = 14,200 µg = 14.2 mg detritus/g d.w. total Macoma sample.

$\frac{14.2 \times 88}{1000}$ = 1.25 µg Zn/g d.w. sample, associated with detritus.

2.27- 1.25 = 1.02 µg Zn/g d.w. sample, incorporated into tissue.

RESULTS

The only metal which appeared consistently at detectable levels in sea water was cobalt, whereas Cr, Eu, and Sc appeared sporadically and no Fe or Zn were detected. As shown in Table 1, the amount of Co released to the sea water by oiled and non-oiled sediment did not differ.

Table 1. Co content of filtered seawater (µg x 10-s/ml).

Days Exposure	Oiled	Non-oiled
2	5.1	4.5
8	4.15	no sample
15	6.0	5.5

The detrital contents, as calculated from the scandium levels of the samples, indicated that the clams fed during the first two days of direct exposure to detritus, and the net amount of food in their digestive tracts declined thereafter as shown in Table 2 and Figure 1. On day 2, in the absence of oil, 25.6 mg/g of body d.w. was composed of detritus. In the presence of oil only one half as much food was ingested initially and it was lost. at a greater rate than in the non-oiled clams.

In the filtered compartment of the aquarium to which no oil had been added, less than one tenth as much scandium labelled material, assumed to be detritus, was taken up initially as in the non-oiled detritus compartment. The Sc level in clams in the filtered compartment declined more slowly and irregularly than that in clams in the detritus compartment. Clams receiving filtered water from oiled detritus took in about as much food as the non-oiled controls.

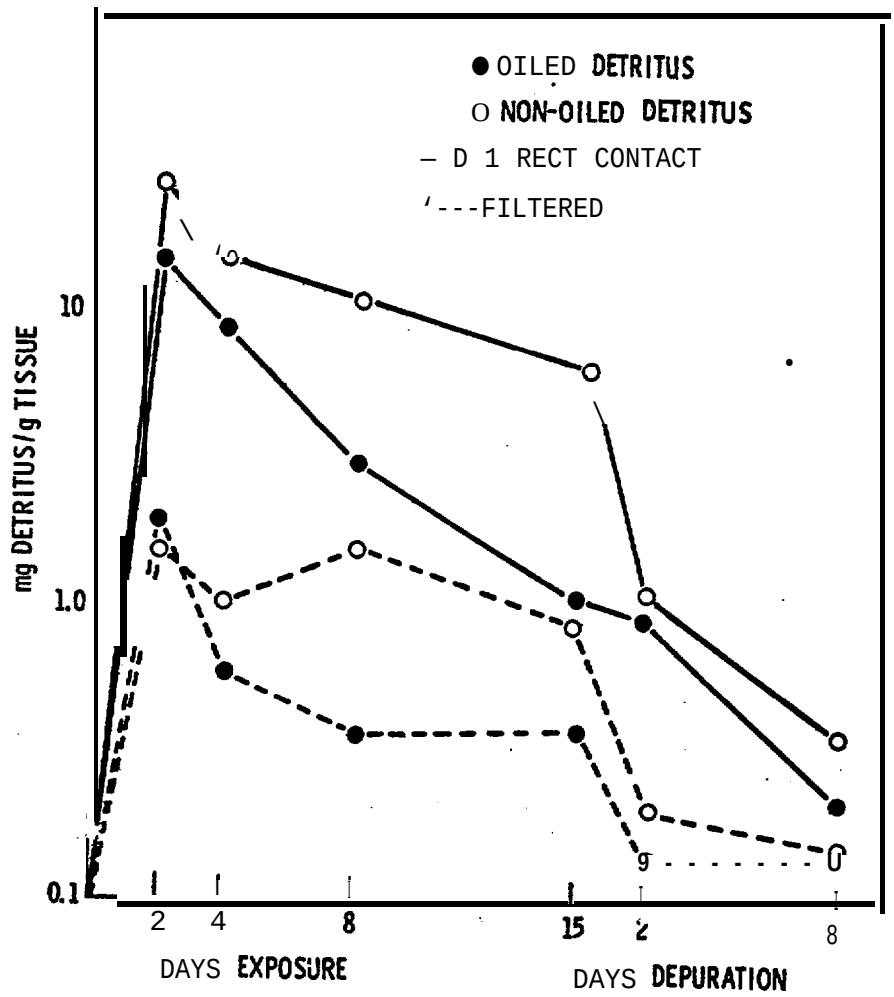

Figure 1. Detritus (mg/g) ingested by Macoma inquinata.

Following the transfer of clams to tanks containing non-labelled detritus, part of the radioactive material was quickly lost from the gut, but a fraction, on the order of one to ten percent of the originally ingested material, remained at the end of eight days.

Table 2. Calculated weight of detritus (mg/g d.w.) in Macoma inquinata.

Days Exposure	Non-oiled	Oiled	Non-oiled (filtered)	Oiled (filtered]
2	25.7	13.5	1.53	1.99
4	14.2	8.31	0.96	0.57
8	10	2.97	1.52	0.37
15	6.2	1.07	0.88	0.38
Days Deputation				
2	1.1	0.88	0.22	0.14
8	0.37	0.24	0.15	0.14

Table 3 shows the amounts of Zn and Co per g d.w. incorporated into Macoma tissue. Chromium was detected in only three clam samples. The levels of Eu present in the total samples differed from the amounts calculated to be in the detritus by less than 10^{-2} µg/g in all but one case. probably, these small differences were artifacts produced by the random nature of the gamma-emitting process, and europium, like other rare earths, is not absorbed from the gut. Similarly, the apparent net uptake of iron was so irregular and represented such a small proportion of the iron present in the detritus that it does not provide firm evidence that any iron was taken into the tissue.

Figures 2 and 3 show the amounts of Co and Zn incorporated into Macoma tissues during two weeks exposure to labelled detritus and one week deputation. Those organisms "which received their metal through the water column or on very fine particles incorporated nearly identical amounts whether oil was present

Table 3. Zn and CO (µg/g d.w. tissue) incorporation into Macoma inquinata exposed to oiled and non-oiled detritus.

	Zn				Co			
Days Exposure	NO	O	NOF	OF	NO	O	NOF	OF
2	0.24	0.29	0.26	-	0.024	0.059	0.009	0.019 (0.009)
4	1.02	1.38	0.32	-	0.070 (0.038)	0.112	0.025	0.043
8	1.18	1.09	0.572	0.95	0.175 (0.038)	0.108 (0.033)	0.049 (0.088)	0.043 (0.022)
15	3.41	1.38	1.05	0.87	0.140 (0.051)	0.067	0.042	0.054
Days Deputation								
2	2.23	0.62	0.94	1.01	0.216	0.130	0.043 (0.066)	0.026 (0.026)
8	2.10	0.99	1.10	1.32	0.133	0.080	0.037 (0.038)	0.046

O = oiled
NO = not oiled
F = filtered
- = no metal present in sample
figures in () = concentrations in shells

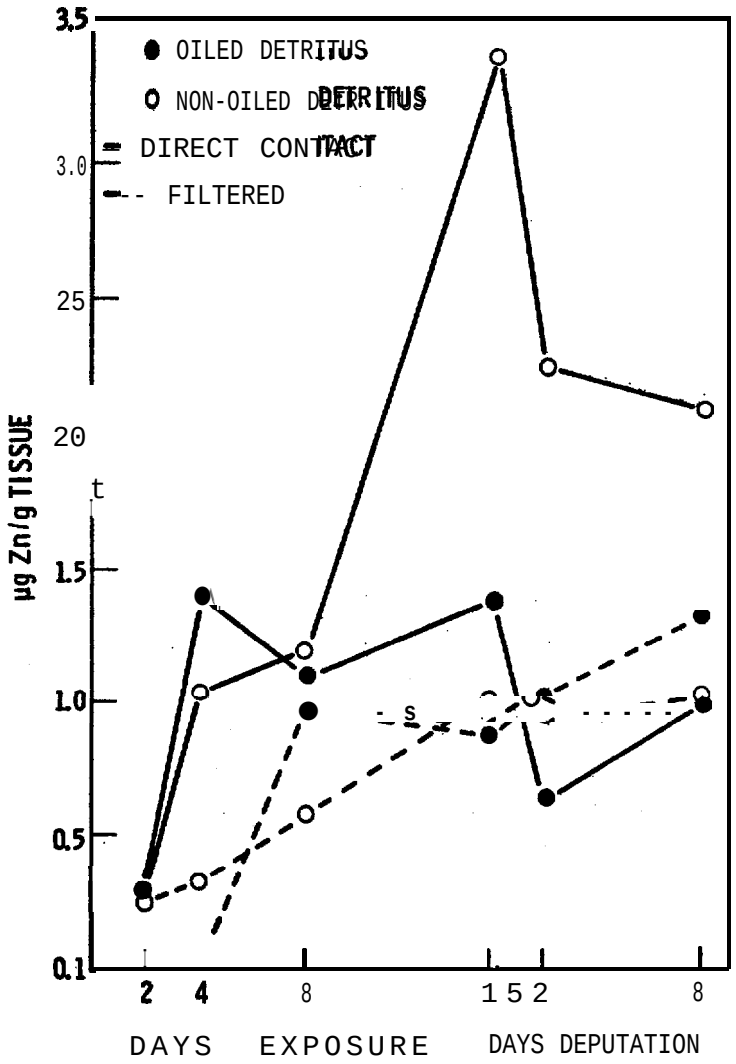

Figure 2. Incorporation of **radiolabelled** zinc into **Macoma** tissue.

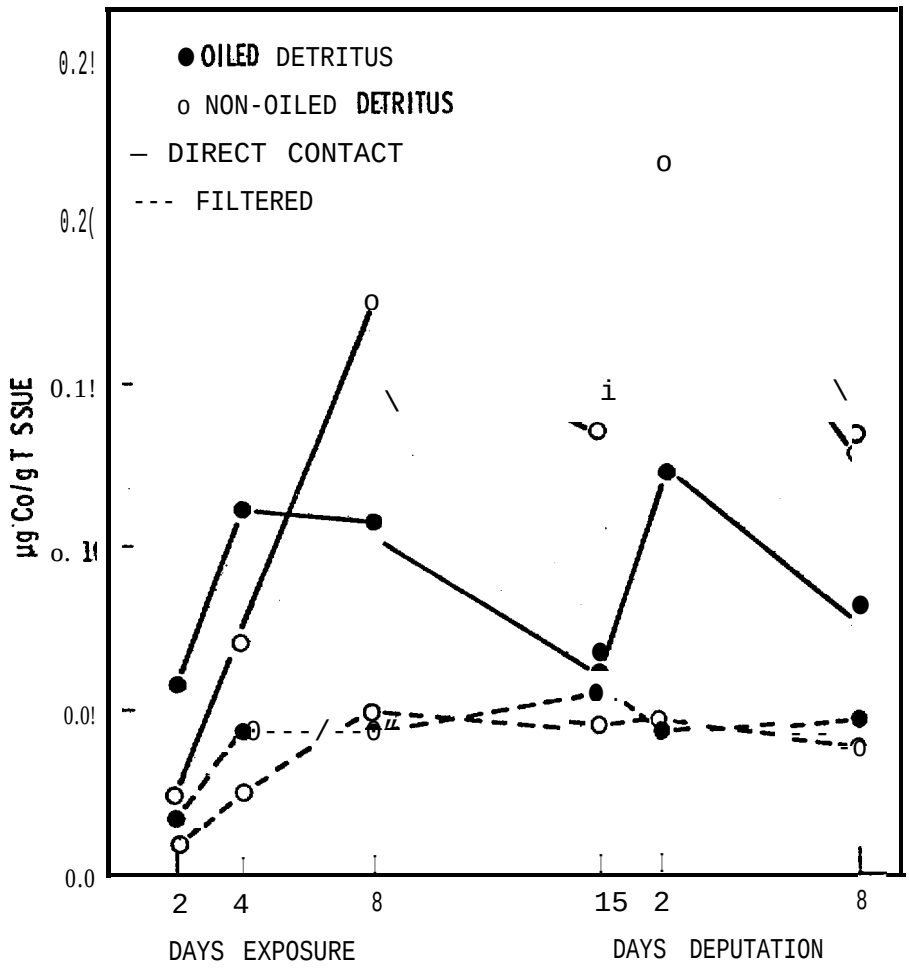

Figure 3. Incorporation of **radiolabelled** cobalt into **Macoma** tissue.

or absent. Those which had direct access to detritus incorporated the same amounts early in the exposure period, but later the oiled animals took in less. This difference is probably due to the fact that less oiled than non-oiled detritus was ingested and, therefore, a smaller amount of labelled metal was available for absorption across the walls of the intestinal tract.

The only metal to appear consistently in the shells was cobalt. Its concentration there was of the same order of magnitude as in the clam meat. There was no indication that more cobalt was taken into the shells from oiled than from non-oiled detritus.

DISCUSSION

The detritus on which the clams fed in this experiment was approximately the same material they ingest in nature. There is, therefore, no reason to believe that the concentrations of metals in their food were any higher during than before the experiment or to expect a net increase in metal concentration in the controls, i.e., those animals exposed to non-oiled detritus. The fact that labelled Zn and Co appeared in the controls indicates that a more or less rapid exchange takes place between the metals in the tissue and those in the food or water. The persistence of some labelled metals during the period of deputation may be caused by the retention of old material in the animals' gut after their transfer to clean aquaria.

The normal tissue zinc concentration of Macoma is about 200 ppm. The cobalt concentration is not known, but probably resembles that of other bivalves from unpolluted waters, which is on the order of 0.5 ppm. Thus, the amount of labelled zinc and cobalt taken in by non-oiled Macoma in two weeks,

presumably replacing metals lost to the environment, amounts to about 1% and 30%, respectively, of their normal metal pool. If hydrocarbons enhanced the rate of uptake of metal from detritus, then oil-exposed animals would be expected to accumulate metals from the detritus at a faster rate than that at which they lost them to the environment and so to exhibit a greater increase in radioactivity than is found in the non-oil exposed clams. This was not the case, since the net amount of radioactivity incorporated into the tissues of oil-exposed clams over time was less than that in the tissues of clams exposed to non-oiled detritus. This reduction in metal uptake by tissue, however, does not imply that PHCS reduce the ability of Macoma to absorb metals from detritus, but that PHC reduces the feeding rate of exposed clams.

There seem to be no grounds for believing that exposure to 1000 ppm PHC either increases or decreases the rate at which Macoma absorbs metals, except through a reduction in the rate of food intake. This conclusion is supported by the fact that in the filtered compartment of the aquarium, where the absolute differences between food intakes of oiled and non-oiled animals were less and where more of the metals were probably taken in via the water column, the amounts incorporated by the two groups were quite similar. The results of this experiment therefore indicate that, while the presence of crude oil in sediment may affect the clams' condition through its effect on their feeding behavior, it is not likely to increase the risk of heavy metal toxicity to the population. However, PHC contamination of sediment could alter patterns of metal transfer in the marine benthic community and change the food web due to changes' in feeding behavior.

ACKNOWLEDGEMENTS

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IV. SUBLETHAL EFFECTS OF PETROLEUM HYDROCARBON. CONTAMINATION OF SEDIMENTS

IV-A:

EFFECTS OF PRUDHOE BAY CRUDE OIL CONTAMINATED SEDIMENTS ON PROTOTHACA STAMINEA (MOLLUSCA:PELECYPODA): HYDROCARBON CONTENT, CONDITION INDEX, FREE AMINO ACID LEVEL

by

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ABSTRACT

Protothaca staminea and Macoma inquinata were exposed to sediment contaminated with 1237 ppm Prudhoe Bay crude oil in the field. Eighty-five percent of the Protothaca and 17% of the Macoma survived 54 days exposure. Body burdens of saturate and di- and tri-aromatic hydrocarbons were less than 2 ppm and quite variable. The condition index of Protothaca was reduced by 6% by exposure to oil. The level of free glycine in mantle, gills and adductor muscle did not change significantly, but the taurine level fell, leading to a decrease in the taurine:glycine ratio. It was concluded that Protothaca, a filter feeder, is affected less severely by oil pollution than Macoma, a detritivore, perhaps because the feeding activity of filter feeders is inhibited less.

INTRODUCTION

Roesijadi and Anderson (1979) reported that exposing the deposit-feeding bivalve, Macoma inquinata to oil-contaminated sediment resulted in reduced survival, reduced condition index (CI), and reduced levels of free amino acids (FAA), principally glycine, in the gills, mantle, and adductor muscle. Roesijadi, Anderson, and Blaylock (1978) had found this species to accumulate substantially larger amounts of diaromatic hydrocarbons from sediment than the filter-feeding bivalve, Protothaca staminea. An experiment has been undertaken, using more severe exposure conditions, in order to examine a wider range of petroleum hydrocarbon (PHC) compounds taken up by the two species and to compare the physiological impact of exposure on P. staminea with that on Macoma.

METHODS

P. staminea, M. inquinata, and the sipunculid Phascolosoma agassizi, in addition to sediment, were collected from the protected side of a sand spit at the mouth of Sequim Bay, Washington State, U.S.A. The animals were kept under running seawater at about 10°C and 30 parts per thousand (°/oo) salinity for six days before use. The sediment used was that from the animal collection site which passed through a 6 mm mesh screen. Ninety-eight ml of Prudhoe Bay crude oil was emulsified in a blender with two 500 ml portions of sea water. The oil and one ℓ sea water were added to 42 kg of sieved sediment in a cement mixer and mixed for one hour. The oiled sediment was flushed three times with sea water while in mesh bottomed trays, 25 x 47 x 12 cm in size. Unoiled control sediment was similarly treated. Sediment samples were taken for determination of initial total hydrocarbon content by IR spectrophotometry (API, 1958). Initial control groups of clams were taken for condition index

and free amino acid determinations by the methods of Roesijadi and Anderson (1979), and the remainder were placed in the sediment trays. Thirty Protothaca were placed in each of three control and three oiled trays. Thirty Macoma and 30 Phascolosoma, a detritivorous sipunculid worm, were placed in one oiled tray, and 20 Macoma and 30 Phascolosoma in one control tray. On January 11, 1978 the trays were placed in the low intertidal zone in the area from which the animals and sediment had been collected.

On March 6, 1978 the trays were removed, and the total hydrocarbon "content of the sediment was determined by IR spectrophotometry. Surviving animals were counted, left overnight in running sea water to allow their intestinal contents to be cleared, and frozen at -65°C. Two Macoma, three Protothaca, and samples of the sediment were taken for analysis of individual hydrocarbon compounds by glass capillary gas chromatography (Warner, 1976). Condition indices and free amino acid levels of control and exposed Protothaca were determined by the methods of Roesijadi and Anderson (1979). Condition Indices were calculated according to the formula:

$$C.\ I. = \frac{\text{g ash free dry weight}}{\text{cm shell length}} \times 1000$$

RESULTS

Initially the oiled sediment contained 1237 ± 112 ppm hydrocarbons (n = 4), and the control sediment 10 ± 11 ppm (n = 2). After 54 days exposure 850 * 17 ppm (n ="4) remained in the contaminated trays. In the control trays 82 out of 90 Protothaca (91%), all 20 Macoma (100%) and 11 out of 30 Phascolosoma (37%) remained. In the trays containing oiled sediment 77 out of 90 Protothaca (85%), but only 5 out of 30 Macoma (17%) and no Phascolosoma were

recovered alive. The more mobile worms may have migrated from the oiled environment, but the missing clams presumably died. As Table 1 shows, in control animals the level of PHC compounds is below the limit of detection. The PHC content of exposed animals was less than 2 ppm and highly variable within each species. The mean content of the two Macoma tested was twice that of the three Protothaca examined. The most consistent and marked contrast between the two species was the naphthalene level, which was an order of magnitude higher in Macoma.

A difference, significant at the .01 level, was found between the mean condition indices of exposed and control Protothaca (Table 2). There was a decrease of similar proportion among control animals between the beginning and end of the exposure period, but it was not statistically significant. Analysis of the free amino acids in gill, mantle, and adductor muscle (Table 3) showed reductions, significant at the .01 level, in the contents of alanine, histidine, and leucine in controls at the end of the experiment, compared to initial controls. The total FAA content however rose over this period, due to a 29% increase in the level of taurine. Comparison of control and exposed animals taken after 54 days in the field showed highly significant reductions only in the levels of lysine and taurine. The latter was quite variable, being almost absent in some exposed clams. There was a highly significant difference in total amino acid content between the two groups, two thirds of which was accounted for by the decline in the mean level of taurine.

DISCUSSION

In June, 1977 Roesijadi and Anderson (1979) exposed M. inquinata to sediment initially containing 1144 ± 47 ppm hydrocarbons. Their exposure period lasted 38 days, during which time the petroleum hydrocarbon (PHC)

Table 1. Glass capillary gas chromatographic analyses of petroleum hydrocarbons content (µg/g) in Macoma and Protothaca tissue and sediment after 54 days exposure to oil-contaminated sediment.

	Macoma			Protothaca				
		Exposed			Exposed			
	Control	I	II	Control	I	II	III	Sediment
Saturates nC_{12} - nC_{28}	<.047	.381	.514	<.196	<.948	.181	<.154	35.57
Pristane	-*	.020	.009	<.018	.002	.009	-	2.085
Phytane		.023	.011			.010	-	1.170
Naphthalene		.021	.032		.001	<.001	<.009	0.347
2-MN		.022	.048		.022	.020	.023	1.401
1-MN		.023	.081		.025	.024	.019	0.761
1 Ethyl + 2 Ethyl Naphthalene	-	.023	.181		.012	.001	.018	0.300
2,6 + 2,7 DMN	-	.023	.092	<.014	.076	.0(J?	.018	0.653
1,3 + 1,6 DMN	-	.023	.135	<.015	.039	.035	.030	0.580
1,7 DMN		.023	.021		.022	.026	.009	0.822
~,4+2,3+l,5B}~N-		.024	.096		.057	.002	.024	0.486
1,2 DMN		.026	.052		.034			0.162
Phenanthrene	-	.010	.102		.017	.070	.036	0.114
C1 Phenanthrenes	-	.014	.225		.044	.007	.064	0.028
C2 Phenanthrenes	-	.014	.271		.051	.009	.074	0.311
Total Diaromatics	-	.208	.638	<.043	.288	.111	.153	5.512
Total Triaromatics	-	.038	.598		.112	.026	.174	0.453
Total PHC Identified	<.074	.670	1.770	.206	1.350	.337	.481	44.791

* To reduce confusion in reading the table, all values <0.008 have been removed. The variability in the detection threshold is a result of differences in the recovery of internal standards and the sample weight.

MN = methyl naphthalenes
DMN = dimethyl naphthalenes
C1 = methyl
C2= dimethyl

Table. 2. Condition indices of initial control, field control, and exposed Protothaca staminea.

	$\bar{x} \pm$ S.E.	n
Initial Control	19.3 ± .36	10
Field Control - 54 days in clean trays	18.2 ± .20 n.s.	59
Exposed - 54 days in contaminated trays	17.1 ± .17**	64

n.s. Not significantly different at .10 level from initial control.
** Significantly different from field control at .01 level when means are compared by student's t test.

Table 3. Free amino acid concentration in gills, mantle, and adductor muscle of Protothaca staminea (μmoles/g) after 54 days exposure to oil-contaminated sediment.

	Initial Control	Field Control	Field Exposed
Alanine	9.47 ± 1.48	6.47 ± 2.23††	4.01 ± 2.3*
Arginine	2.84 ± 0.56	2.75 ± 0.85	1.92 ± 1.31
Aspartic Acid	1.30 ± 0.65	1.15 ± 0.47	0.56 ± 0.50*
Glutamic Acid	3.89 ± 1.57	3.26 ± 0.88	1.99 ± 1.32*
Glycine	26.7 ± 6.5	21.9 ± 7.8	18.33 ± 12.6
Histidine	0.21 ± 0.06	0.11 ± 0.03††	0.34 ± 0.70
Isoleucine	0.27 ± 0.05	0.24 ± 0.10	0.15 ± 0.10
Leucine	0.43 ± 0.10	0.24 ± 0.11††	0.31 ± 0.16
Lysine	0.34 ± 0.12	0.37 ± 0.11	0.21 ± 0.11**
Methionine	0.08 ± 0.0	0	0
Phenylalanine	0.31 ± 0.08	0.22 ± 0.07†	0.22 ± 0.17
Proline	0.26 ± 0.08	0.16 ± 0.10†	0.10 ± 0.09
Serine[1]	1.17 ± 0.30	0.91 ± 0.18†	0.93 ± 0.67
Threonine	0.36 ± 0.13	0.28 ± 0.08	0.21 ± 0.13
Tyrosine	0.28 ± 0.06	0.25 ± 0.08	0.21 * 0.22
Valine	0.37 ± 0.07	0.33 ± 0.10	0.23 ± 0.15
Taurine	33.6 ± 10.6	43.3 ± 17.1	18.36 ± 13.05**
Total	79.94 ± 17.96	85.56 ± 26	48.08 ± 24.5**
Taurine:Glycine	1.23 ± 0.28	2.04 ± 0.54	1.38 ± 0.78

† Significantly different from initial control at .05 level; student's t test.
†† Significantly different from initial control at. .01 level; student's t test.
* Significantly different from field control at .05 level; student's t test.
** Significantly different from field control at .01 level; student's t test.
[1] SeFine, glutamine, and asparagine co-chromatograph.

content declined to 364 ± 66 ppm. These conditions were obviously less severe than our winter exposure of 54 days, during which the PHC level remained above 800 ppm. The lesser severity is reflected in the fact that in the earlier experiment 59% of the exposed Macoma survived while only 17% did so in this experiment. Earlier, Roesijadi, Anderson and Blaylock, (1978) had exposed Phascolosoma agassizi for 60 days to sediment containing 887 ppm PHC at the beginning and 420 ppm at the end of the period. Their experimental animals survived and remained in their trays, while the sipunculids exposed here either died or migrated out. This contrast also indicates the greater stressfulness of the present exposure. Nevertheless, even under the present harsher conditions 85% of the exposed Protothaca survived.

The number of Macoma surviving the winter exposure was too small to allow comparisons to be made of sublethal physiological effects with those on winter exposed Protothaca. These Protothaca apparently were affected less intensely than the surviving summer exposed Macoma of Roesijadi and Anderson (1979). Thus the average condition index of Macoma inquinata exposed to oil-contaminated sediment for 38 days in June, 1977 was 16% lower than that of control animals (Table 4). By contrast, the condition index of Protothaca exposed in January 1978 was only 6% less than that of the controls. Since it is not likely that the shell length of any individual animal changed significantly during the course of the experiment, the condition indices are directly proportional to the ash free dry weights. The decline from control levels by exposed Protothaca was only one third as large as that exhibited by exposed Macoma. This indicates that the former were not forced to draw on their stored reserves for nutrition to as great an extent as the latter. That the absolute value of the CI of control Protothaca is substantially higher

Table 4. Survival and condition index of Macoma and Protothaca after exposure to oil-contaminated sediment.

	Survival (%)	Condition Index	PHC Content Of Sediment (ppm) Initial	Final
Macoma				
June				
Control	92	8.92 ± 0.18		
Exposed	59	7.46 ± 0.28** (16. 3% decrease)	1144 (± 47.3)	364 (± 66.4)
Macoma				
January				
Control	100			
Exposed (54 days)	17		1237 (± 112)	850 (± 17)
Protothaca				
January				
Control	91	18.2 ± 0.20		
Exposed (54 days)	85	17.1 ± 0.17** (6% decrease)		

**Significantly different from control at .01 level by student's t test.

than that of control *Macoma* is due to the fact that *Protothaca* is wider in proportion to its length than *Macoma*. Therefore, in animal's of equivalent nutritional status a given unit of shell length corresponds to a larger volume of flesh for *Protothaca*.

The changes found in free amino acid levels in the tissues of *Protothaca* differ from those reported in other bivalve species under stress. Roesijadi and Anderson (1979) found a pattern in *Macoma* resembling the stress syndrome described by Bayne *et al*. (1976) in *W edulis*. In their exposed animals the average level of glycine was reduced to 62% of that in the controls, while the taurine level stayed constant, leading to an increase in the taurine: glycine ratio. Jeffries (1972) reported a drop in glycine level in stressed *Mercenaria mercenaria* accompanied by an increase in taurine levels, again leading to an elevated taurine:glycine ratio.

In the *Protothaca* exposed in this experiment the reverse was found. The mean glycine level of exposed clams did not differ significantly from that of controls, but the mean level of taurine was reduced by 58%, leading to a decline in the taurine:glycine ratio. Table 5 compares the relevant data. The significance of the decline in taurine is not clear at this time, since its only known role in the metabolism of marine invertebrates is to function in osmoregulation. There is no reason to suspect any difference between the salinity level of the water surrounding the oil-exposed and the control groups.. However, there is some evidence to indicate that the taurine levels of *Protothaca* tissue may fluctuate as a result of conditions not related to stress or osmoregulation. Roesijadi (1979) reported that holding *Protothaca* in the laboratory for 26 days under constant temperature and salinity resulted in a significant decrease in the taurine level of the gills while the glycine levels remained constant. Stress, in the form of addition of up to 155 µg/ℓ

Table 5. Free amino acid content (μmoles/g) of Macoma and Protothaca.

	Macoma		Protothaca	
	(June, 38 day exposure)		(January, 54 day exposure)	
Amino Acids	control	Exposed	Control	Exposed
Glycine	70.25 ± 4.51	43.56 ± 4.94	21.9 ± 7.8	18.33 ± 12.6 n.s.
Lysine	0.59 ± 0.04	0.41 ± 0.04	0.37 ± 0.11	0.21 ± 0.11
Threonine	1.16 ± 0.05	0.87 ± 0.07	0.28 ± 0.08	0.21 ± 0.13 n.s.
Taurine	37.06 ± 1.94	35.08 ± 2.39	43.3 ± 17.1	18.36 ± 13.05
TOTAL	150.57 ± 8.15	110.48 ± 8.24	85.56 ± 26.0	47.0 ± 24.5
Taurine:Glycine	0.54 ± 0.04	0.89 ± 0.12	2.04 ± 0.54	1.38 ± 0.78

n.s. No significant difference, other differences significant at .01 level.

of Na hypochlorite led to a significant decrease in the concentration of glycine but not of taurine (Roesijadi, 1979).

In view of these data, which indicate that taurine levels may change in this species for unknown reasons, it may be more useful to consider the changes in other free amino acids, especially glycine, as indicators of stress. There is only a slight, statistically insignificant, decrease in the glycine levels in the oil-exposed Protothaca. This response, together with the smaller decrease in condition index and the higher rate of survival, all support the conclusion that the suspension feeder Protothaca is less vulnerable to oil-contaminated sediment than the deposit feeder Macoma.

It is difficult to relate this lower vulnerability to a lower body burden of PHC except in the case of naphthalene. Roesijadi, Anderson, and Blaylock (1978) pooled their samples of exposed Macoma and found the level of total diaromatics to be an order of magnitude higher than in exposed Protothaca. If the same procedure had been followed in this work, a qualitatively similar difference would have been found for all aromatics, though of smaller extent. However, one individual exposed Macoma contained lower levels of most hydrocarbon compounds examined than some exposed Protothaca, in agreement with the findings of Roesijadi and Anderson (1979) who found quite low levels (.08 µg/g) of diaromatics in their exposed Macoma. Comparison of body burdens of individual PHC compounds with corresponding levels in the sediment showed no trend toward tissue magnification except in the case of methyl phenanthrene. Other triaromatics and a few diaromatics approached equilibrium with the sediment levels, while most diaromatic and saturate concentrations are one to two orders of magnitude lower in tissue than in sediment.

From these few analyses there was only a slight difference in the level of hydrocarbon accumulation in the tissues of the two species, while there are

clear differences in the severity of physiological impact of oil pollution. Therefore, these data are not sufficient to support the hypothesis that the impact of oil pollution on the survival and physiological state of intertidal bivalves results directly from the toxic effects of hydrocarbon compounds. It is possible that the effects noted are instead the result of a decline in feeding activity induced by the oil. Gilfillan et al. (1977) reported a negative correlation between carbon flux and tissue aromatic HC content in Mya arenaria collected from sites contaminated by an accidental oil spill. Stegeman and Teal (1973) found that Crassostrea virginica reduced its production of faeces and pseudofaeces in proportion to the hydrocarbon concentration in the surrounding water at concentrations between 100 and 400 µg/l. In our laboratory, Augenfeld, Anderson and Crecelius (in preparation) observed a 50% decline in detritus intake by Macoma presented with food tainted with Prudhoe Bay crude oil. Augenfeld (1980, in press) reported an 80% decrease in sediment egestion rate by Abarenicola pacifica, a deposit feeding polychaete, in heavily contaminated sediment. Gordon, Dale and Keizer (1978) noted a decrease of up to 93% in the sediment-working rate of the similar form Arenicola marina in the presence of 275 ppm oil in sediment. The material taken in by filter feeders is in less direct contact with oil associated with sediment than the food taken in by deposit feeders. As a result, Protothaca may have been inhibited in its feeding activity to a lesser degree than Macoma. The enhanced survival and reduced level of stress shown by the former may thus be the direct result of a superior nutritional status.

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IV-B:

EFFECTS OF PRUDHOE BAY CRUDE OIL CONTAMINATION ON SEDIMENT WORKING RATES OF ABARENICOLA PACIFICA

by

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ABSTRACT

Lugworms (Abarenicola pacifica) were exposed to sediment containing 250 to 1000 ppm Prudhoe Bay crude oil. At concentrations of 500 and 1000 ppm the rate of feeding, as measured by faecal production, was reduced by 70%. Smaller control animals turned over more sediment in proportion to their body size than larger ones. Exposure to oil at high levels abolished this difference by greater depression of the rate of faecal production by smaller individuals.

INTRODUCTION

Accidentally spilled petroleum hydrocarbons (PHCs) which impact fine-grained sediments in intertidal zones may remain in situ for periods of years (Mayo et al. 1978; Teal, Burns & Barrington, 1978), resulting in long-term effects on local populations (Krebs & Burns, 1977). One factor which may contribute to this persistence is the existence of anoxic conditions at depths of more than 1 cm below the surface of these habitats (Teal & Kanwisher, 1961; Pamatmat, 1968; Hylleberg, 1975). ZoBell (1964) has reported that PHCS are degraded at much slower rates in anaerobic sediments than under aerobic conditions.

The burrowing and feeding activities of certain organisms transport sediment from the lower anoxic areas to the surface, where aerobic microbes can metabolize hydrocarbons more rapidly. One such organism which occurs in high densities in fine-grained sediment along the shores of the North Pacific is the sedentary polychaete worm, Abarenicola pacifica Healy & Wells. Hobson (1967) has calculated that a population of A. pacifica located in False Bay, San Juan Island, Washington State, U.S.A., could move all the sediment in the upper 10 cm of the area they inhabit to the surface in a little more than two years. Their activity could aid in the recovery of intertidal zones from the effects of oil pollution if they can continue to feed in contaminated sediment.

Gordon, Dale & Keizer (1978) reported that exposure in the laboratory to No. 2 fuel oil, Venezuelan Bunker C., South Louisiana crude, Kuwait crude oil, or to sediment which had been impacted by an accidental spill of Bunker C led to reductions of 51% to 82% in sediment-working rates of Abarenicola marina L. This species is found in sandy beaches on both sides of the North Atlantic and is closely related to A. pacifica. Gordon et al., found that direct contact with sediment containing oil at concentrations as low as 153 ppm for as little as 5 days caused some of their worms to surface, and some died at concentrations of 275 ppm.

In our laboratory A. pacifica has been found to tolerate direct contact with sediment containing 1000 ppm of Prudhoe Bay crude oil (PBC) for more than three weeks. It therefore seemed possible that the mud-dwelling Pacific species is more tolerant of oil contamination than the sand-dwelling Atlantic species. An experiment was designed to learn whether contamination of their habitat with various levels of PBC would reduce the amount of sediment which A. pacifica transports to the surface. This experiment made use of the fact

that the worm deposits its faeces in easily recognizable coils around the entrance to its burrow.

MATERIALS AND METHODS

Forty specimens of A. pacifica and 12 kg sediment were collected from the high intertidal region of an almost enclosed lagoon adjacent to Sequim Bay, Washington State, U.S.A. The upper 10 cm of the sediment consisted of very fine-grained, semi-liquid mud, and a layer of firmer fine-grained sand lay beneath. In the laboratory the worms were kept in sediment under running sea water at 10°C and 30°/00 salinity.

Sediment (2.5 kg wet wt.) consisting of equal volumes of the mud and sand strata, was placed in each of four cylindrical metal containers of 3-liter capacity. Three different volumes of PBC (.625, 1.25 or 2.5 g) were added to three containers, and the contents of each, including the unoiled control, were stirred 6 minutes with a motor-driven impeller. Previous trials with the dispersion of radio-labelled material have shown this time period to a be adequate for thorough mixing. The resulting mixtures were injected into 40 pieces of tygon tubing, 40 cm long and 17 mm id. The tubes were bent into U-shapes and placed in racks under running sea water for 20 hours to allow the more toxic low molecular weight components of the oil to wash out. Remaining sediment was placed under running sea water as a reserve supply.

One worm was placed in each tube, and plastic trays, 64 mm on a side, were placed around the ends of the tubes. At 24-hour intervals the water levels in the tanks were lowered and the faeces produced by each worm were collected with a stainless steel spatula from the trays and from the surface of the sediment within the tubes. At intervals of several days sediment from the appropriate reserve supply was added to the tubes to replace consumed

material. The faeces were dried in air for 24 hours and weighed. Preliminary studies indicated that this time period was long enough to achieve a constant weight. All calculations of faecal cast production were based on the mean daily dry weight produced by each individual worm.

After 11 days of exposure, the sediment and worms were removed from the tubes. The worms were rinsed with sea water, blotted dry, weighed and frozen. Samples of the sediment were taken from tubes at each treatment level, frozen, and later analyzed for total hydrocarbon content by IR spectrophotometry.

RESULTS

The level of PHC in the sediment changed little during the exposure period. The worms' native substrate used in the experiment has a fairly high endogenous content of organic material which absorbs IR radiation at the same wavelengths as hydrocarbons. The HC content of sediment at the three treatment levels, when corrected for interference by the organic content of the control sediment, was between 80 and 100% of the amount originally added. The actual concentrations of PBC to which the worms were exposed lie somewhere between the levels originally added and the levels measured at the end of the experiment. For the sake of convenience, these levels will be referred to as those originally added, viz. 250 ppm (low), 500 ppm (medium), and 1000 ppm (high).

All the control worms survived, but two of them left their tubes and burrowed into detritus at the bottom of the tank, where their faeces could not be collected. Mortality was slightly higher in the exposed groups, and some signs of behavior stress were observed. When the faeces were collected, at 24-hour intervals, some of the worms exposed to medium and high levels of PBC were seen to extend their posterior segments from the burrows

and slowly move them through the water. This behavior, which would obviously be maladaptive under natural conditions, was never observed in the controls or in the field. Feeding behavior was apparently depressed during the first two days of exposure, to a greater extent in the higher concentrations. During the remaining nine days the frequency of defecation, based on 24-hour observation periods, was slightly, but not significantly, lower in the exposed animals. Data on faecal production was therefore taken from this nine-day period, as being more representative of the long-term effect of oil exposure.

A strong negative correlation was found between faecal production per unit body weight and body weight. The slope of the regression line relating these parameters was calculated according-to the formula

$$b = \frac{\Sigma xy - \frac{\Sigma x \, \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

and found to be -2.56 for the control and low-level exposure groups and only -0.50 for the two higher-level exposure groups (Figure 1). The degree of scatter around the regression lines was less in the exposed groups than in the control. Mean faecal production per unit body weight was moderately depressed in low-level exposed groups.

The regression of dry faecal weight, unadjusted for body weight, on body weight, was not significant within any treatment group; i.e., it could not be statistically demonstrated that the total amount of sediment turned over by an individual worm was affected by the weight of the worm. The mean produced faecal weight is markedly reduced by exposure to hydrocarbon levels above 500 ppm. Pairwise comparison of the control with each treatment group by

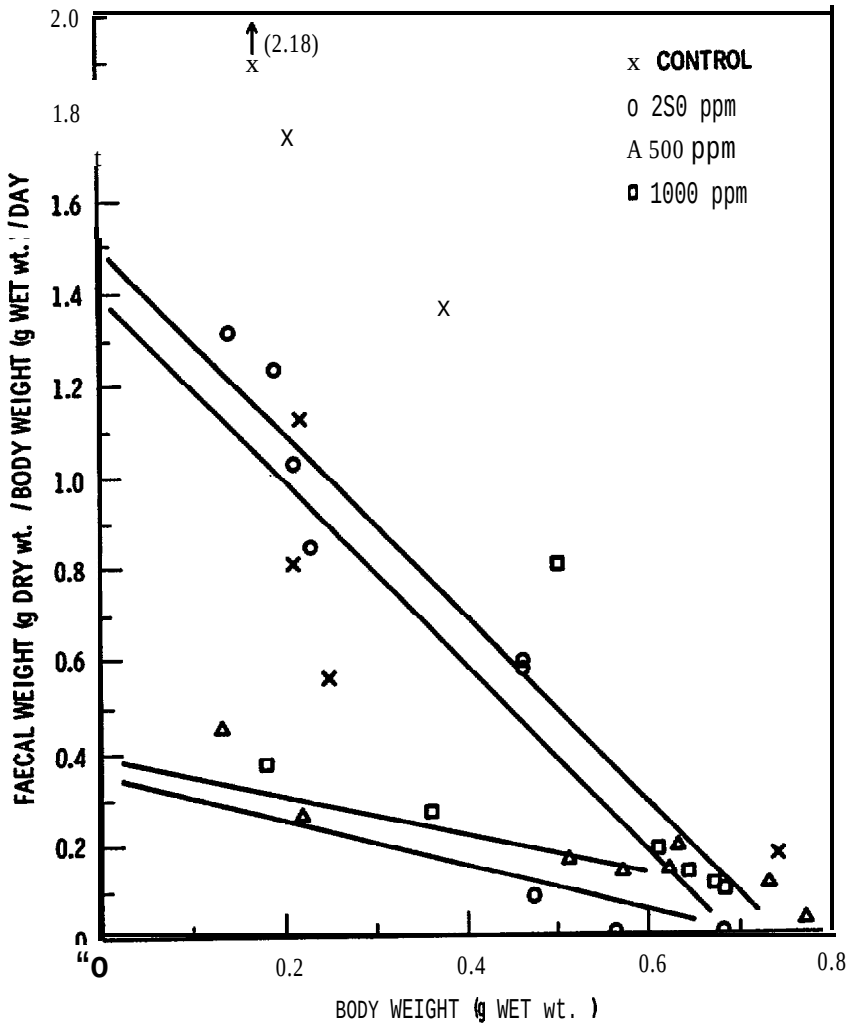

Figure 1. Regression of **Faecal** Weight Per Unit Body Weight on Body Weight.

Control Y = 1.839 - 2.56 x; r = -.653; **n** = 8

250 ppm Y = 1.600 - 2.56 x; **r** = -.941; n = 9

500 ppm Y = .449 - **.50** x; r = -.921; **n** = 8

1000 ppm Y = .438 - .5o x; **r** = -.885; **n** = 7

the Newman-Keuls test showed no significant difference between it and the low-exposure level, while the mean production rate at the medium- and high-exposure levels differed significantly at the .05 level from that of the controls.

DISCUSSION

Exposure to oil reduces the degree of scatter around the regression lines relating faecal production per unit body weight to body weight of A. pacifica, and at levels above 500 ppm greatly reduces the slope of the lines (Figure 1). Both of these effects may be attributed to a greater impact of exposure to oil on the smaller and more active animals. Among control worms weighing less than 0.25 g, faecal production per unit body weight varied between 0.9 and 2.1 g feces/g worm/day. Production by worms in this size class ranged from 0.84 to 1.3 g/g worm/day under exposure to a low level of oil. At the higher oil levels values for the smaller worms fell to between 0.37 and 0.45 g/g worm/day. By contrast, the maximum production of worms weighing more than 0.6 g was reduced only from 0.2 g/g worm/day in control animals to .08 g/g worm/day at the higher exposure levels. Exposure to oil clearly leads to a greater reduction in feeding activity at the lower end of the size range than at the upper end. The individual variability of production is lessened by a greater decrease in the maximum rate achieved by the smaller animals than in the minimum rates.

These data indicate some of the effects of hydrocarbon pollution on the survival and activity of the worms. However, measures of faecal production per unit body weight such as these, and those reported by

Gordon et al. (1978), are less useful in predicting the effect of the affected worms on sediment turnover, since predictions based on them would require information on the size distribution of the exposed population, which might not be available. Fortunately, the effect of the negative regression of faecal production on body weight is cancelled out by the increasing body weight itself, with the result that within each treatment group the regression of faecal weight, uncorrected for body weight, on body weight is not significant at the .05 level.

The somewhat surprising conclusion is that under uniform conditions and within the weight range examined, which was .14 to .74 g, the size of the worms has no significant effect on the average dry weight of faeces produced per day. Therefore, if suitable control populations were available for comparison the defecation rate of A. pacifica could be used as an indicator of a particular level of environmental stress, even if the size distribution of the worm population is not known.

The results also suggest that if pollution is not too severe, Abarenicola may continue to turn over the equivalent of its own wet body weight per day in sediment. However, high concentrations of oil may reduce the sediment-working rate of surviving worms by as much as 70%. This effect, together with any mortality due to the environmental pollution, would substantially retard the tranportation of sediments to the surface. If these effects are found under actual field conditions, it is possible that sufficiently high levels of oil will retard the rate of sediment recovery by reducing the feeding behavior of ecologically significant species. Measurements of the food intake of deposit-feeders and detritivores may be a valuable means of evaluating effects of oiled substrate, as Roesijadi & Anderson (1979) have shown that condition index and free amino acid content of the deposit-feeding clam, Macoma, decreased during field exposure to oiled sediment.

ACKNOWLEDGEMENTS

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Iv-c:

EFFECTS OF PRUDHOE BAY CRUDE OIL IN SEDIMENT ON *ABARENICOLA PACIFICA* IN LABORATORY AND FIELD EXPERIMENTS

by

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Introduction

Hydrocarbon pollutants are particularly persistent in fine grained intertidal sediments (Mayo *et al.*, 1978; Teal *et al.*, 1978), and, once introduced, may affect local populations for extended periods (Krebs & Burns, 1977; Sabo & Stegeman, 1977). In view of this vulnerability it would be advantageous to identify responses on the part of organisms typically found in such habitats which could provide early warnings of a deterioration in environmental quality. Behavioral responses are often sensitive indicators of changes over short time frames, but observations of organisms buried in mud presents obvious difficulties. There is however one form of behavior taking place within the sediment which can be quantified by an observer at the surface. This is the burrowing and feeding activity of organisms which deposit their feces around the entrance of permanent burrows.

One such group of organisms are the Arenicolidae, or lugworms. Several characteristics of these sedentary worms make them potentially valuable as indicators of environmental quality. They are relatively non-motile, survive well in the laboratory, and are found in large numbers in sensitive habitats. In addition, their pattern of feeding and respiratory behavior increase their

potential exposure to substances both in the substrate surrounding them and the water column above them, since they ingest large amounts of sediment and use undulating body motions to pass large volumes of water over their gills.

Casual observations in our laboratory suggested that the rate of burrowing of Abarenicola pacifica, a species typical of muddy tidal flats, was reduced by hydrocarbons in its environment. Gordon, Dale and Keizer (1978) reached similar conclusions for Arenicola marina, a larger, related form, typical of sandy intertidal areas. Their work was carried out in the laboratory for periods of up to two weeks. The work described here included similar experiments using Abarenicola and was extended to explore the possibility of using burrowing rates of lugworms as an indicator of pollution levels in the field over longer periods of time.

If a reduction in burrowing rates is reflected in reduced food intake, a decline in nutritional status might be expected. In this study we examined the level of free amino acids in the tissues as a possible indicator of nutritional status.

Methods

Animals and sediment were collected from an intertidal mud flat adjacent to Sequim Bay, Washington State, U. S. A. (48°5' N, 112°3'W). The same area was used for field exposures. Table 1 indicates the grain size distribution of the site. As over 25% of the particles present were less than 50 µm in diameter the sediment would be classed as a silty sand. Immediately after collection the sediment was mixed with Prudhoe Bay crude (PBC) oil for one hour in a cement mixer or stirred for shorter periods with a motor driven impeller. Control sediment was mixed in the same way without the addition of

TABLE 1

PARTICLE SIZE DISTRIBUTION IN TIDE FLAT HABITAT OF *ABARENICOLA PACIFICA*.

SIZE	>5.0 MM	2,0-5,0	1,0-2,0	1.0-0.5	0,5-0,05	0,05-0s002	<0.002
%	0.2	Lo	2.2	16.2	52,2	26,6	1.6

oil. After mixing, the oiled sediment was placed in running sea water for 24 hours to allow part of the lighter, more toxic, fractions to be flushed out before the worms were placed in it.

Experiments designed to measure changes in body composition in the laboratory were carried out in fiberglass trays with mesh bottoms. For measurements of burrowing/feeding rates in the laboratory forty artificial burrows, consisting of U-shaped tygon tubes, were set up. Ten contained clean sediment, and ten contained sediment to which Prudhoe Bay crude oil at original concentrations of 250, 500 or 1000 ppm had been added. One A. pacifica was placed in each burrow. Feces were collected daily from plastic trays surrounding the ends of the tubes, dried, and weighed. The experimental procedure is described in more detail in Augenfeld (1980).

To expose worms to known levels of petroleum hydrocarbons under field conditions, the bottoms were removed from 800-ml polyethylene beakers and replaced with 800 micron mesh Nitex screen. One worm was placed in each beaker with uncontaminated sediment or sediment mixed with PBC. The beakers were set into the collection area of the tide flat at such a depth that the surface of the sediment in the beakers was level with the surface outside. Feces were collected daily from the surfaces of the beakers and dried to constant weight.

A second field exposure was set up, using controls and an intermediate concentration of oil, i.e., 400 ppm, in two conditions. Sixteen animals were placed in sediment containing fresh PBC. The sediment-oil mixture in these beakers was only flushed in sea water for 24 hours before the worms were added. A second set of 16 was placed in sediment containing PBC which had been weathered for 24 days by exposure to sunlight and flowing sea water beneath it.

Amino acid determinations were carried out on sections of body wall musculature weighing ca 100 mg. They were minced and shaken with 80% ethanol (1 mℓ/10 mg tissue) for24 hours. The extract was centrifuged and the total amino acid content of the supernatant was determined by the method of Clark (1964).

Results

Laboratory exposure: burrowing rates

After eleven days under flowing seawater the low, medium and high oiled sediment contained approximately 250, 400, and 800 ppm oil, respectively, as measured by infrared (IR) spectrophotometry. One, two, and three worms, respectively, died in the low, medium, and high level exposure system, while all the controls survived (see Table 2). Animals exposed to 400 to 500 ppm oil were seen at times to extend the posterior portion of their body from their burrows and move them slowly through the water. Animals exposed to 800 to 1000 ppm did so more often. This behavior was never observed among worms exposed to sediment containing no or low levels of oil.

Control worms began depositing feces at once, and after a lag period of two days, exposed animals did so too. As has been reported earlier (Hylleberg, 1975) the pattern of defecation was irregular, with pauses of varying lengths between intervals of activity. On the average, approximately one set of castings was produced per two days.

As Table 3 shows, A. pacifica exposed to sediment captaining more than 400 ppm PBC reduced their burrowing rates on the average by over 75%. Exposure to 250 ppm resulted in a 36% reduction. These effects were not exerted to the same extent on all size ranges in the population. Among control and low level exposed animals the smaller individuals produced a

TABLE 2

EFFECTS OF PBC ON SURVIVAL AND BEHAVIOR OF A. PACIFICA

		EXPOSURE LEVEL		
	CONTROL	LOW	MEDIUM	HIGH
SURVIVAL	10/10	9/10	8/10	7/10
LEFT TUBES	2/10	0	0	0
TAIL EXTENDED FROM TUBES	0	0	6	12
CAST PRODUCTION* FIRST 2 DAYS	415	315	2	1
CAST PRODUCTION* NEXT 9 DAYS	5	4.9	4.6	4.5

* MEAN NUMBER OF CASTS/DAY PRODUCED BY ALL SURVIVING WORMS

TABLE 3

EFFECT OF PRUDHOE BAY CRUDE OIL ON FECAL PRODUCTION OF *A. PACIFICA*: LABORATORY EXPOSURE

	EXPOSURE LEVEL			
	CONTROL	LOW	MEDIUM	HIGH
MEAN BODY WEIGHT ± STANDARD ERROR	.32 ± 159	,32 ± .14g	.17g	,52 ± ,16g
MEAN FECAL DRY WEIGHT/WEEK	1.75 ± 98	1,12 ± ,77	,06	,077 ± .06
MEAN FECAL DRY WEIGHT/ MEEK/ 9 BODY WEIGHT	6,93 ±2.10	4,41 ±1.68	1,26 ± .84	1s9 ± ,84
REGRESSION OF MEAN FECAL WEIGHT ON BODY WEIGHT	',31 N.S.	',37 N.S.	+.012 N.S.	+, 012 N.S.
REGRESSION OF MEAN FECAL WEIGHT/g BODY WEIGHT ON BODY WEIGHT	-2,56	-2.56	-,51	-,50

larger volume of burrowed material per unit body weight than did larger animals. As Figure 1 shows, all individuals weighing less than 0.4 g produced more than 0.5 g dry weight in feces per g body weight per day, in the control and low oil sediments, while all individuals in this size range produced less than 0.5 g dry weight feces per g. body weight per day, when exposed to 400 or 800 ppm oil. Among worms weighing more than 0.5 g the differences in burrowing rates between exposed and control individuals was not significant at the .05 level. Within each treatment group fecal production per unit body weight grew smaller as body weight grew larger. As these two trends cancelled each other out, fecal weight per se did not change significantly with changing body size (cf. Augenfeld, 1980).

Laboratory exposure: amino acid content

At the time that the effect of Prudhoe Bay crude oil on body wall amino acid content was tested, a heavy fungal growth appeared on the surface of oiled sediment in the laboratory and, to a lesser degree, on control sediment. Since the presence of this material, which covered the entrances to the burrows, might impose an additional stress on the worms, the test concentration of oil was reduced to 400 ppm. Nevertheless, there was increased mortality among the exposed animals. While all the controls survived, only six out of nine exposed individuals survived one week and only two out of nine survived two weeks. Half of these survivors left their burrows and stayed on the sediment surface. In spite of the obviously severe conditions the free amino acid levels in the muscle did not change significantly after one week, and declined by only 9% after two weeks (see Figure 2).

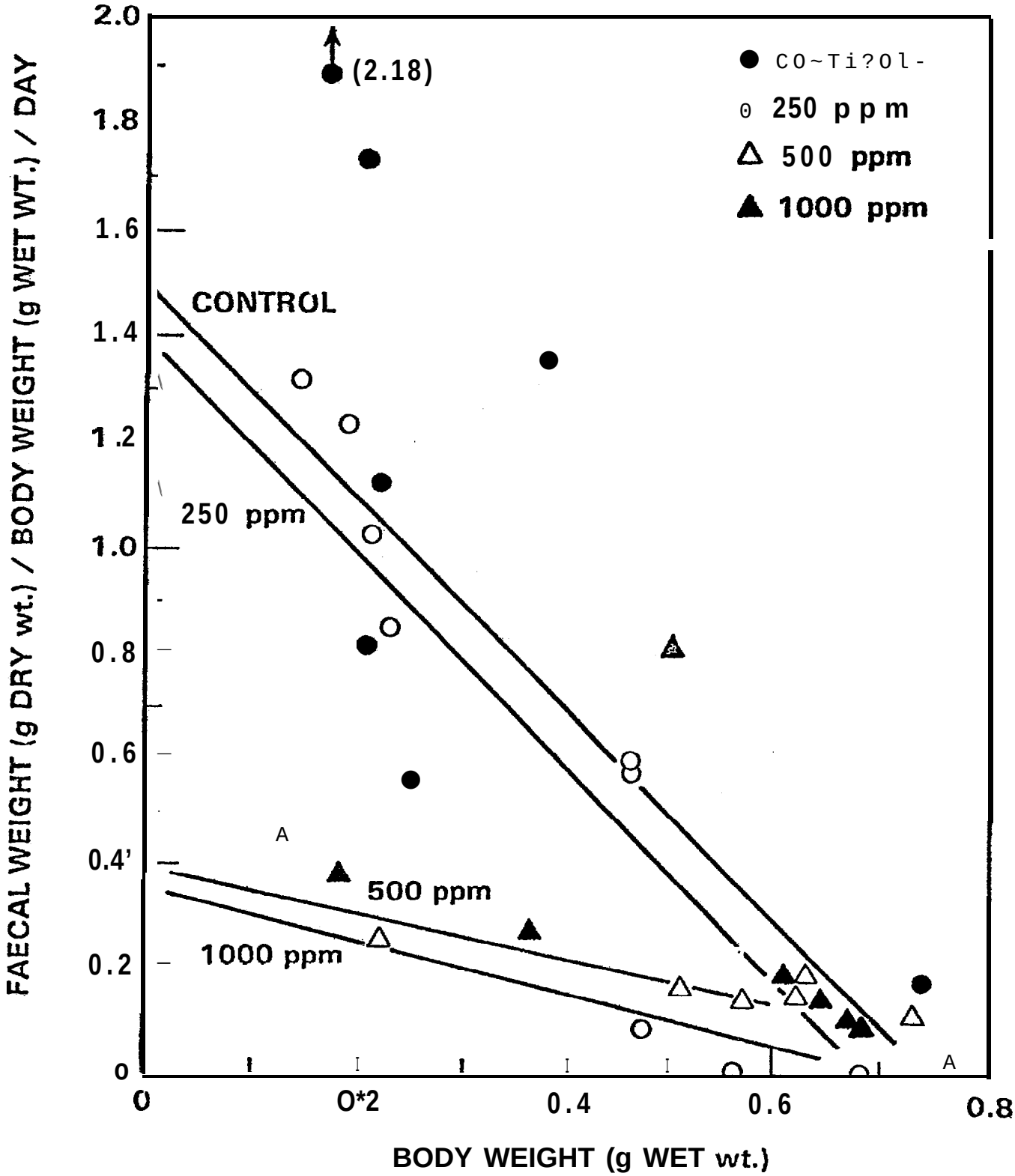


Figure 1. Regression of weight-specific fecal weight on body weight: Effect of Prudhoe Bay crude oil in sediment.

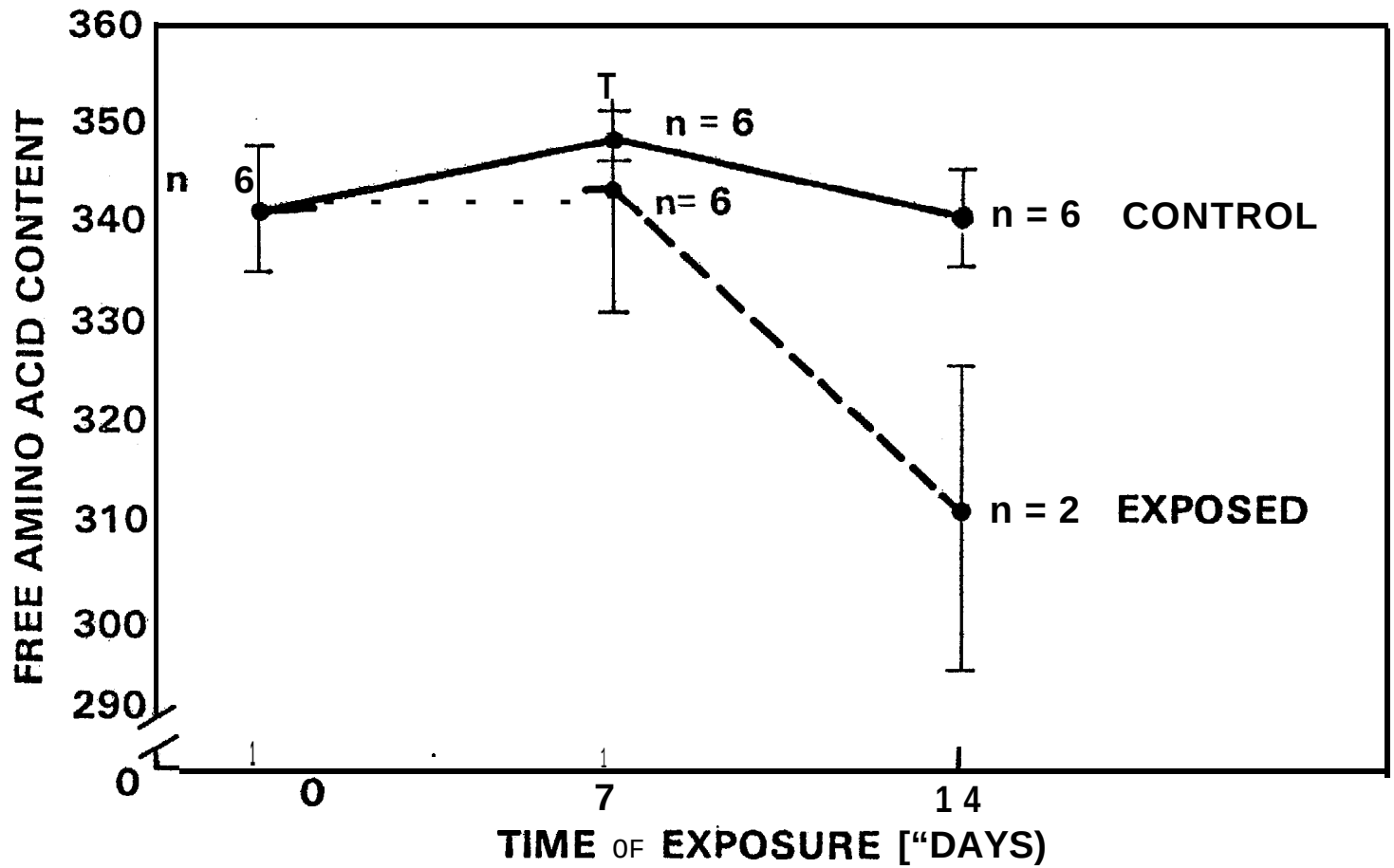


Figure 2. Free amino acid content of body wall muscle of Abarenicola pacifica (µm/g wet wt.) following laboratory exposure to sediment containing 400 ppm Prudhoe Bay crude oil.

Field exposure: burrowing rate

The experiments designed to measure burrowing rates in the field were carried out in September, 1978, three months after the lab exposures. At this time the mean weight of the Abarenicola in the collecting area was 0.? g, a size at which no significant effect had been found in the lab. Since it was not known in advance whether exposure to oiled sediment in the field would have more severe or less severe effects than exposure in the lab, a maximal concentration of 1000 ppm and a minimal concentration of 200 ppm were employed. Over a five week period the concentration of oil in the sediment declined to 600 and 100 ppm.

Twelve individuals were used at each level. No feces appeared in the beakers containing the higher level of pollutant. After two and a half weeks these beakers were examined and found to contain no worms. Partially decayed remains of worms were found in some beakers, indicating that they had. died in situ rather than having migrated. A new set of animals was placed in the beakers, on the assumption that the previous group might have been killed or driven out by the presence of toxic low molecular weight compounds such as benzene or naphthalene, which tend to be washed out of sediment sooner than heavier molecules (compounds). The second set of animals also produced no feces, and at the end of five weeks these beakers contained no worms. In the control beakers containing stirred but unoiled sediment, seven out of twelve animals survived and remained, and in the low level exposed group six out of twelve did so.

As is summarized in Table 4, burrowing activity of the worms in the control beakers began at a slow rate, compared to that of the laboratory population. It increased steadily through the five week period of observation until, during the fifth week, it was five times as high as during the first

TABLE 4

EFFECT OF PRUDHOE BAY CRUDE OIL CONTAMINATION ON FECAL PRODUCTION OF A. PACIFICA: FIELD EXPOSURE.

	DRYWEIGHT FECES/WORM/WEEK				
	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
CONTROL	,305 * ,0919	,661 ± .193g	.910 ± ,209	.89 ± ,249	1,53 ± ,34s
EXPOSED	,168 ± ,069	. 685 ± ,127	.87 ± .23	,56 ± ,16	.98 ± .14

week and not significantly different from that of the control group in the lab. The group exposed to sediment containing a low level 'of petroleum hydrocarbon (PHC) contamination turned over, on the average, 55% as much material as the control group during the first week. During the next two weeks these animals matched the controls, and during the last two weeks of observation they fell behind again to 64% of the control levels. None of these differences however was statistically significant at the .05 level.

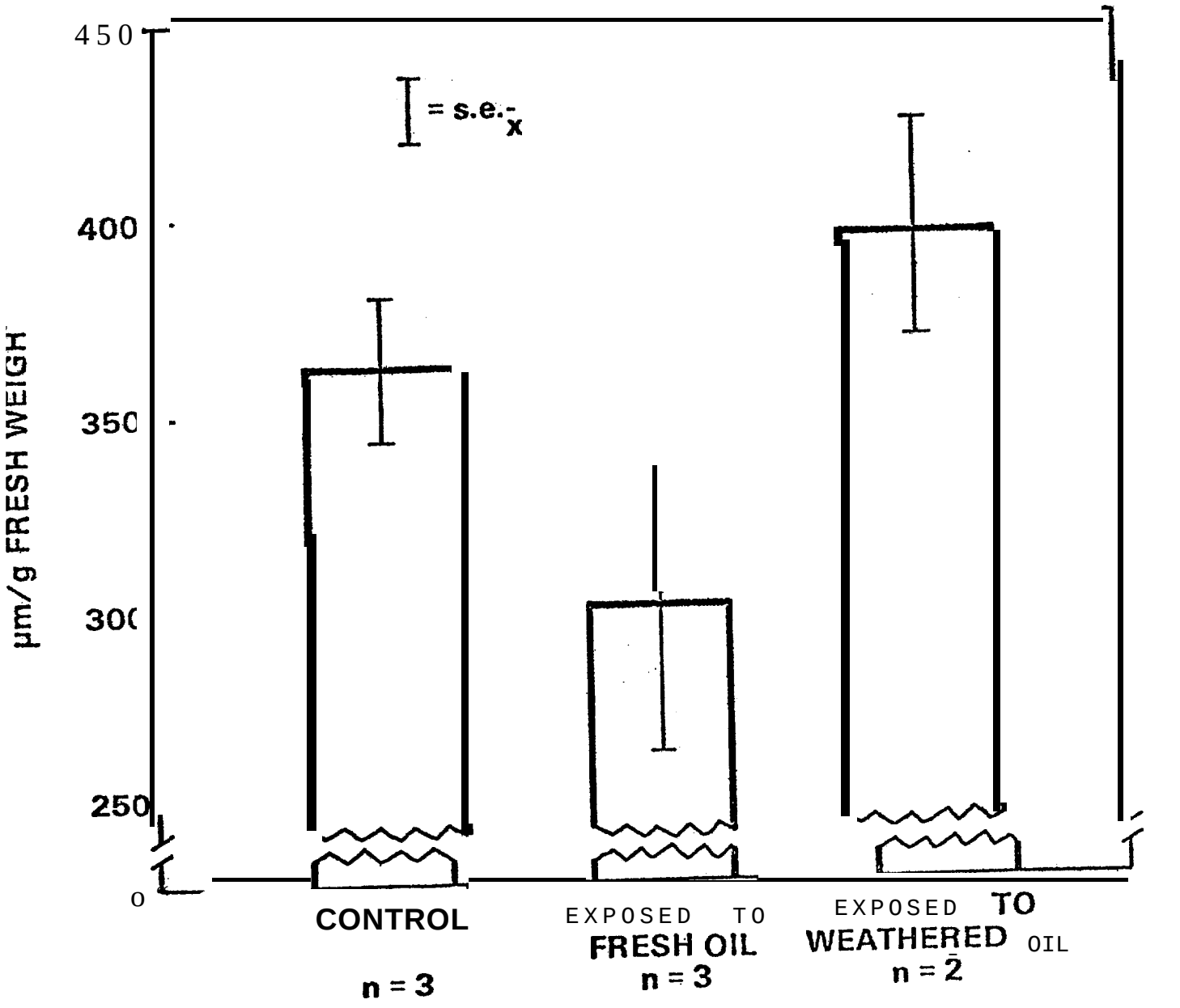
Field exposure: amino acid content

The conditions affecting the organisms during this exposure were evidently more stressful than those present during the earner field experiment, since only three controls, three animals exposed to fresh oil, and two exposed to weathered oil survived. Further, very few feces were produced by the surviving animals, and most of these were in the form of loose grains of material which was readily dispersed in the water, instead of solid coils, which could be collected. Therefore it was not possible to make quantitative measurements of burrowing rates. As Figure 3 shows, the mean muscle free amino acid contents of the two worms which survived exposure to weathered oil was slightly higher than that of the controls. This in turn was as high as the level found in animals collected directly from their normal habitat. The content of worms exposed to fresh oil was 18% lower than in the controls.

Discussion

While the results obtained in these experiments do not rule out the use of burrowing rates as indicators of stress on Abarenicola populations, they do indicate a need for caution in extrapolating from laboratory results to conditions which might be found in the field, and in planning for and

Figure 3. Free amino acid concentrations in A. pacifica body wall muscle: field exposure to 400 ppm Prudhoe Bay rude oil in sediment.



interpreting the results of field observations. The most striking contrast between laboratory and field results was the difference in mortality of worms exposed to sediment containing more than 600 ppm oil. In the lab 70% of the exposed animals survived. In the field there was 100% mortality. The difference may have resulted from the additional stress on the field population of coping with periodic emersions during low tides. May (1972) described a behavior pattern by which A. pacifica draw air bubbles into their burrows from which oxygen may be absorbed, even if fresh supplies of oxygenated water are not available. If the presence of high levels of oil in the sediment affects this behavior pattern, as it affects burrowing rates in the laboratory, it may interfere with an even more vital function than feeding and so cause the death of the organism. In the laboratory situation, where the supply of oxygenated water is more constant, the behavior pattern described. by May would be less critical for survival. Another possible reason for the higher mortality rate in the field lies in the response seen in the lab in which part of the worms' bodies are extended from the burrows. Such a position would make the animal more vulnerable to predation by fish or shore . . birds. On the other hand, the more vigorous flushing action of the natural tidal cycle apparently resulted in a greater reduction in PHC concentration in the sediment than took place in the lab. Perhaps it was a result of this that the worms exposed to the lower concentration of oil in the field attained a mean burrowing rate that matched that of the controls, after the first week of exposure.

Another cause for caution pointed up by these results lies in the highly irregular pattern of fecal production over time by individual control animals, both in the lab and in the field. In order to separate any "signal" resulting from a response to pollution from the "noise" generated by the animals' own

patterns, it will be necessary to continue observations over longer periods of time. If the responses are of a transient nature, as appears to have been the case in the field burrowing rate experiments a larger number of animals will have to be observed.

Nevertheless, these results may be taken as at least a preliminary suggestion that lugworm burrowing rates may be developed into a useful monitoring tool. At the least the rates could be used in laboratory studies as indicators of the ability of suspected pollutants of sediments to affect the behavior of benthic infaunal organisms.

Their usefulness as a measure of effects in the field, however, may be restricted, at least in the case of PHCS, to a narrow range of concentrations. As we have seen, the effects at low concentrations maybe subtle enough to require the use of large numbers of organisms to detect their significance, while at substantially higher concentrations the effects are lethal. This finding is in accord with observations made along the coast of Brittany after the wreck of the Amoco Cadiz. There it was reported (Chasse, 1978) that in the five km of coast line nearest to the wreck, populations of Arenicola marina suffered heavy mortality, while at sites only slightly more distant they appeared to be unaffected. It may be concluded that in order to use lugworm burrowing rates as measures of effects in the field without requiring unrealistically large numbers of animals, more information will have to be gained on normal variations in these rates and on the background factors affecting survival.

There is little indication from these experiments that muscle free amino acid content in these animals is affected to a substantial degree by PHC in the sediment. It is true that a slight reduction was seen after periods of exposure of two weeks or more, but these were in survivors of populations that

had suffered heavy mortality. Some of these survivors were under severe enough stress to have left their burrows, which would certainly result in their death under natural conditions. It therefore seems unlikely that a significant reduction in mean amino acid concentration would be observed at lower levels of stress. While such reductions have been suggested as parameters to be monitored in bivalve mollusks (Jeffries, 1972; Bayne et al., 1976; Roesijadi and Anderson, 1979), their significance in at least one polychaete has not been established.

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V. EFFECTS OF WEATHERING ON OIL

V-A:

CHANGES IN THE VOLATILE HYDROCARBON CONTENT OF PRUDHOE BAY CRUDE OIL TREATED UNDER DIFFERENT SIMULATED WEATHERING CONDITIONS

by

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ABSTRACT

Changes have been determined in the concentrations of volatile saturate and aromatic hydrocarbons in Prudhoe Bay Crude oil (PBC) weathered under three different simulated environmental conditions. A combination of light and water spray upon the surface of the oil produced the largest relative decreases in volatile saturate and most aromatic hydrocarbons. After 24 days, detectable amounts of monoaromatic hydrocarbons were absent in all three weathered oils as were the saturate hydrocarbons from C_8 to C_{10}. Retention of aromatic hydrocarbons appeared to be related to molecular weight, as enrichments of triaromatics (phenanthrenes) were observed in weathered PBC relative to the original crude oil. These data are discussed with respect to effects resulting from spilled oil impacting benthic organisms residing in intertidal and shallow subtidal communities.

INTRODUCTION

The potential environmental impact to benthic marine organisms and the intertidal environment from spilled oil may depend on the extent to which oil has been exposed to the action of various dissipative factors prior to impact. Major processes which contribute to the weathering of oil include evaporation, dissolution, photo-oxidation, emulsification and biodegradation. (National Academy of Science, 1975). Water soluble fractions rich in aromatic hydrocarbons produced from the dissolution of oils have produced both acute and chronic effects in marine organisms in the laboratory (Neff et al, 1976; Anderson (1977), Malins (1977), Anderson et al. (1978), Anderson et al. [1979). Oil spilled offshore, which impacts near-shore benthic habitats and intertidal sediments would contain reduced levels of these components and, therefore, have the potential of producing different levels of effects on marine organisms, relative to fresh crude oil. There is, therefore, a need to produce quantities of weathered oils to serve as source materials for experimental studies directed toward predicting the environmental effects of weathered oils.

We have designed a research program to produce and characterize weathered Prudhoe Bay Crude oil (PBC), which could subsequently be used by biological investigators in experiments. Analyses were designed to describe changes in the volatile saturate and aromatic hydrocarbons of PBC oil weathered under three different simulated environmental conditions. By altering water agitation and sun exposure, the contribution made by these processes could be evaluated. It is hoped that these data will provide a base for possible correlations of hydrocarbon compositional changes of weathered oils with biological effects observed in future investigations.

MATERIALS AND METHODS

Three large volume tanks measuring 1.6 m in diameter and 0.9m deep (2 m^2 area and 1,830 1 volume) were each layered with 20 1 of Prudhoe Bay crude oil. Each tank received flowing seawater that was maintained at a constant level by an external standpipe.

Each tank simulated a different weathering condition:

Tank #1: To simulate weathering under violent weathering conditions inflow water was sprayed upon the surface of the oil (without a sun shield).

Tank #2: To simulate weathering under calm conditions in the presence of sunlight, a slow flow of seawater was injected from below the oil slick.

Tank #3: To reduce the effects of sunlight (photo-oxidation of hydrocarbons), a system similar to tank #2 was prepared with a sun shield over the oil.

During the 24-day weathering period, several environmental parameters were measured at frequent intervals. Air and water temperatures, salinity,

flow rates and light intensity measurements were taken to aid in characterizing the conditiens.

Three replicate 25 ml oil samples were taken from each tank at days 1, 2, 4, 8, 16, and 24. These samples were placed in small vials that were completely filled with oil, wrapped with foil to exclude light, and sealed with teflon-lined caps before refrigeration. Each sample vial was washed in CCl_4 and dried with nitrogen gas before use. At termination (24 days) l-gallon samples were taken from each tank and immediately shipped to Alaska for use by other investigators under National Oceanic and Atmospheric Administration contract.

Samples of the original and weathered oil were shipped on ice from the Sequim Marine Laboratory to the Pacific Northwest Laboratory, Richland, Washington, for hydrocarbon analyses. Samples (6-100 mg) of oil were chromatographed according to the method of Warner (1976) with the following modifications: fifteen grams of silica gel (Grace Davison Chemical Company, 100-200 mesh) were used to separate the oil into saturate (eluted with 40 ml in hexane) and aromatic (eluted with 86 ml of 20% CH_2Cl_2 in hexane) fractions. The fractions, collected in 40ml con"ical tubes, were concentrated under a stream of nitrogen without the aid of external heat, ● transferred to 5 ml conical vials and concentrated to 1 m"l. An internal standard (2,6,10-trimethyldodecane for saturate fraction, hexamethylbenzene for aromatic fraction) was added to each sample, and the samples were analyzed by capillary gas chromatography. Individual hydrocarbons were separated and quantitated on a Hewlett Packard 5840A gas chromatography employing 30 meter OV-101 glass capillary columns operating at 65° with an initial 4-minute hold and then programmed at 4°min to 250° Data were corrected based on the recovery

data of aliphatic and aromatic hydrocarbon standards. Typical recoveries were 82-111% for saturate hydrocarbons (C_{12} to C_{24}) and 84-89% for aromatic hydrocarbons (naphthalene to dimethylphenanthrenes).

The significance in the differences in the concentrations of hydrocarbons and hydrocarbon classes between tanks was determined by comparing calculated t values based on standard deviation of the difference to a table value of t with two degrees of freedom at a $P = 0.05$.

RESULTS

Several weathering parameters monitored during the course of the experiment are summarized in Table 1.

Water and air temperatures varied little over the 24-day period of weathering, as did salinity. The intensity of ambient light over the tanks (1 and 2) ranged from a mean of 2.5 x 10^5 Lux at noon to about 1 x 10^5 Lux at 8am and 4pm. Since we attempted to enhance agitations of the oil layer in tank 1 by injections of water streams from above, the flow rate was approximately 3 times (27 l/rein) that of the other two tanks. This agitation in tank 1 did indeed produce a weathered product different from the other oils, as a brown "chocolate mousse" was apparent by about 10 days of exposure. This material was strikingly different from the other products, which appeared as the original oil except for the increased viscosity observed in tank 2. When samples of the 3 products were subjected to centrifugation (8,000 RPM for30 min.) separation of 2 or 3 layers was observed. Tank 1 material separated into 3 distinct layers with a mousse layer over water which

TABLE 1
PARAMETERS MEASURED DURING THE COURSE OF THE WEATHERING EXPERIMENT

	$\bar{x}$ + S.D.	N	Range Max imum	Minimum
Air Temperature (C) Randomly taken during day	12.20 ± 1.65	16	15.5	10.0
Water temperature (C)	9.88 ± 0.51	16	10.6	9.0
Salinity (0/00)	30.19 ± 0.36	16	31.0	30.0
Light (Lux) over tanks @ 0800 hr	9*3X104 35.1X104	15	I.75X105	2.2X104
Light (Lux) over tanks @ 1200 hr	2.54X105 ± $9.0X10^4$	10	$3.5X10^5$	1.31X105
Light (Lux) over tanks @ 1600 hr	1.52X105 ± $6.9X10^4$	7	2.7x105	6 .6x104
Flow rate l/rein Tank #1	26.66 ± 4.37	16	30.0	15.0
Flow rate l/rein Tank #2	8.95 ± 0.34	16	10.0	8.5
Flow rate l/rein	8.58:1.93	16	15.0	5.0

was covered by an oil layer. Only the oil and water layers were observed in samples from tanks 2 and 3.

Average relative decreases of saturate hydrocarbons (between C_{12} and C_{26}) in tanks #2 and #3 after 24 days of exposure ranged between 45% and 49% (Table 2). These results are in marked contrast to tank #1 where an average relative decrease of 83% was observed. The saturate hydrocarbons C_8 - C_{10}, although present in the original oil, were not detectable in any of the 24 day weathered oil samples (Figure 1).

The ratios of nC_{17}/pristane and nC_{18}/phytane did not change significantly in the weathered oils relative to the original crude oil (Table 3).

Relative decreases in the concentrations of aromatic hydrocarbons were different from those observed for the saturate hydrocarbons in the three exposure systems. Decreases in aromatic hydrocarbons (naphthalene through 3,6-dimethylphenanthrene) were 37% for tanks #1 and #2 and 9% for tank #3. The tricyclic aromatic hydrocarbons (phenanthrene through 3,6-dimethyl-phenanthrene) appeared to have the greatest persistence with relative enrichments occurring in tank #1 (Table 4). 3,6-Dimethylphenanthrene showed relative enrichment in all three exposure systems. Also reported in Table 4 are concentrations for a variety of mono-aromatic hydrocarbons (toluene through l,2,3,5-tetramethylbenzene). The concentration values reported for these compounds in the original oil were not corrected for recovery, nevertheless, they are reported because, with the exception of the tetramethylbenzenes, none of these compounds was detected in the 24-day weathered oil samples from any of the three exposure systems. These results

TABLE 2

CONCENTRATIONS OF SATURATE HYDROCARBONS IN PRUDHOE BAY CRUDE OIL (PBC) AND IN 24-DAY WEATHERED OIL SAMPLES. CONCENTRATIONS IN MG/GRAM OIL

Compound	Concentration in Original Oil	Concentration in Weathered Oil			Relative Decrease in Concentration(%)		
		Tank #1	Tank #2	Tank #3	Tank #1	Tank #2	Tank #3
C_8	4.20:0.12	c	c	c	100	100	100
C_9	4.42:0.10	c	c	c	100	100	100
C_{10}	4.44:0.35	c	c	c	100	100	100
C_{11}	4.68 ± 0.08	0.15 ± 0.02	0.22 ± 0.01	1.68 ± 0.32	96.8	95.3	64.1
C_{12}	4.62 ± 0.15	0.5470.01	0.9730.02	2.19 ± 0.40	88.3	79.0	52.6
C_{13}	4.46 ± 0.26	0.63 ± 0.02	1.64 ± 0.05	2.38 ± 0.15	85.9	63.2	46.6
C_{14}	4.16 ±0.05	0.69 ± 0.01	1.93 ±0.06	2.26 ± 0.20	83.4	53.6	45.7
C_{15}	3.99 ± 0.22	0.70 ± 0.01	1.99 ± 0.05	2.15 ± 0.24	82.5	50.1	46.1
C_{16}	3.7470.24	0.64 ± 0.02	1.85 ± 0.05	1.99:0.29	82.9	50.5	46.8
C_{17}	3.39 ± 0.42	0.58 ± 0.03	1.75:0.04	1.85:0.26	82.9	48.4	45.4
Pristane	2.07:0.38	0.33 ±0.02	1.0430.02	1.1230.12	84.1	49.8	45.9
C_{18}	2.50 ± 0.24	0.48 ± 0.05	1.38 ± 0.04	1.30 ± 0.23	80.8	44.8	48.0
Phytane	1.05:0.24	0.22:0.01	0.57 ± 0.03	0.58 ±0.04	79.0	45.7	44.8
C_{19}	3.04 ±0.76	0.46 ± 0.01	2.07 ± 0.24	1.7370.10	84.9	31.9	43.1
C_{20}	1.93 ± 0.24	0.38 ±0.02	1.08 ±0.04	0.99 ± 0.17	79.8	44.0	48.7
C_{21}	1.58 ± 0.20	0.33 ± 0.03	1.05 ± 0.27	0.85 ± 0.19	79.1	33.5	46.2
C_{22}	1.86 ± 0.30	0.31 ± 0.03	1.18 ± 0.01	1.24 ± 0.30	83.3	36.6	33.3
C_{23}	1.6570.29	0.30 ± 0.03	1.20 ± 0.12	1.26 ± 0.31	81.8	27.3	23.6
C_{24}	1.27 ± 0.26	0.30 ± 0.04	1.11 ± 0.19	1.00 ± 0.24	76.4	12.6	21.3
C_{25}	1.02 ± 0.55	0.24 ± 0.03	0.79 ± 0.16	0.7170.13	76.5	22.5	30.4
C_{26}	0.76 ± 0.23	0.21 ± 0.02	0.60:0.15	0.53 ± 0.07	72.4	21.1	30.3
Total (C_{12}-C_{26})	43.3924.27	7.3030.10	21.98 ± 0.61	23.9854.27	Avg% 83.1	49.3	44.7

[a] Compounds C_8 - C_{11} were corrected for recovery on the basis of the recovery of C_{12} hydrocarbon and, therefore, are more than likely somewhat low.

[b] $\frac{\text{Concentration in original oil - concentration in weathered oil}}{\text{concentration in original oil}} \times 100$

[c] Indicates compound not detected at the sensitivity level that analyses were conducted.

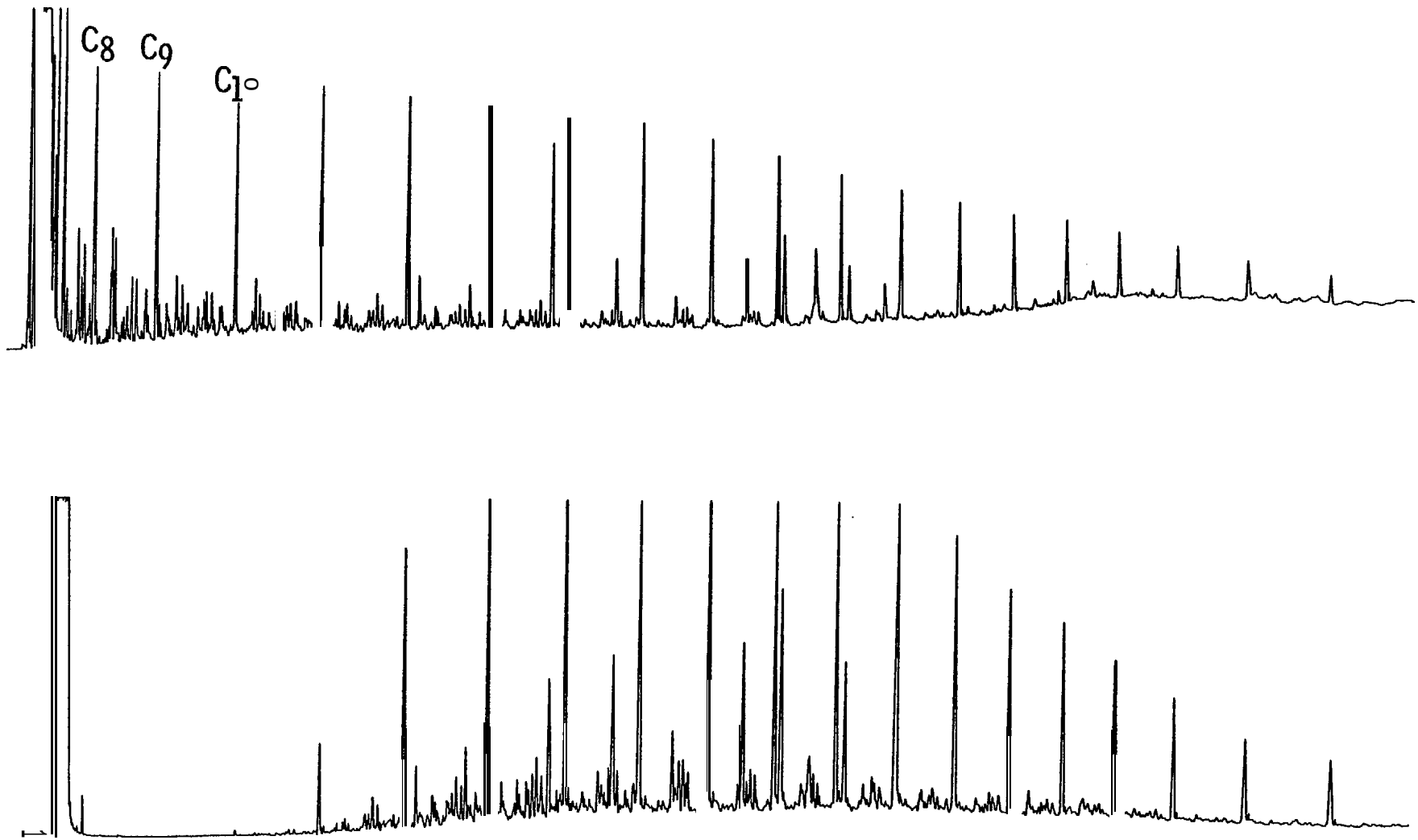

FIGURE 1. Gas capillary chromatograms of saturate hydrocarbon fraction from original Prudhoe Bay crude oil (top) and saturate hydrocarbon fraction from 24-day weathered oil sample from Tank #1 (bottom).

TABLE 3. nC_{17}/pristane and nC_{18}/phytone Ratios from PBC Crude Oil and PBC Oil Weathered in Three Exposure Systems

Ratio	Initial	Final (24 days)		
		Tank #1	Tank #2	Tank #3
nC_{17}/pristane	1.64 ± 0.36	1.75 ± 0.08	1.68:0.03	1.65 ± 0.18
nC_{18}/phytane	2.3S ± 0.25	2.18 ± 0.11	2.42:0.06	2.24:0.19

TABLE 4

CONCENTRATIONS OF AROMATIC HYDROCARBONS IN PRUDHOE BAY CRUDE OIL (PBC) AND IN 24 DAY WEATHERED OIL SAMPLES. CONCENTRATIONS IN MG/GRAM OIL

Compound	Concentration in Original Oil	Concentration in Weathered Oil			Relative Change in Concentration(%)		
		Tank #1	Tank #2	Tank #3	Tank	Tank #2	Tank #3
toluene	0.82 ± 0.08	c	c	c	100	100	100
ethylbenzene	0.56 ± 0.01	c	c	c	100	100	100
m+p-xylene	2.05 +0.04	c	c	c	100	100	100
o-xylene	0.79:0.01	c	c	c	100	100	100
isopropylbenzene	0.16 ± 0.00	c	c	c	100	100	100
l-ethyl+4-methylbenzene	0.29 ± 0.00	c	c	c	100	100	100
l,3,5-trimethylbenzene	0.41 ± 0.00	c	c	c	100	100	100
l,2,4-trimethylbenzene	1.14 ± 0.01	c	c	c	100	100	100
secbutylbenzene	0.14 ± 0.00	c	c	c	100	100	100
methyl-4-isopropylbenzene	0.12:0.00	c	c	c	100	100	100
i ndane	0.67 ± 0.00	c	c	c	100	100	100
l,3-dimethyl-5-ethylbenzene	0.27 ± 0.00	c	c	c	100	100	100
l,2-diethylbenzene	0.24 ± 0.02	c	c	c	100	100	100
l,2-dimethyl-4-ethylbenzene	0.24 ± 0.01	c	c	c	100	100	100
l,2,4,5-tetramethylbenzene	0.38 ± 0.00	0.03 ± 0.00	c	0.25 ± 0.03	d	100	d
l,2,3,5-tetramethylbenzene	0.27 ± 0.00	0.03 ± 0.00		0.18 ±0.02	d	100	d
napthalene	0.92 ±0.01	0.06 ± 0.01	0.16:0.01	0.51 ‘0.08	93.5	82.6	44.6
2-methylnaphthalene	1.63 ± 0.02	0.53 ± 0.07	0.76 ± 0.08	1.34 ± 0.18	67.5	53.4	17.3
l-methylnaphthalene	1.29 ± 0.02	0.48 ±0.06	0.69 ± 0.06	1.20 ± 0.16	62.8	46.5	7.0
l-ethyl+2-ethylnaphthalene	0.48 ± 0.00	0.27 ± 0.03	0.43 ± 0.08	0.51:0.06	43.8	10.4	6.3
2,6+2,7-dimethylnaphthalene	0.69 ± 0.01	0.52 ± 0.06	0.83:0.13	0.94 ± 0.12	24.6	+20 .3	+36, 2
l,3+l,6-dimethylnaphthalene	0.99 fool	0.51 ± 0.06	0.70 +0.05	0.91 ± 0.12	48.5	29.3	8.1
1,7-dimethylnaphthalene	1.10 fool	0.51:0.04	0.77:0.06	0.94 ± 0.12	53.6	30.0	14.5
1,4+2,3+1,5-dimethylnaphthalene	0.80 ± 0.01	0.52 ±0.06	0.35:0.11	0.90 ± 0.12	35.0	56.3	+12.5
1,2-dimethylnaphthalene	0.4030.00	0.23 ± 0.03	0.21 ± 0.01	0.37 ± 0.04	42.5	47.5	7.5
2,3,6-trimethlnaphthalene	0.51 ± 0.07	0.26 ±0.03	0.3930.12	0.4330.04	49.0	23.5	15.7
phenanthrene	0.38 ± 0.05	0.61 ± 0.06	0.3430.05	0.22 ± 0.00	60.5	10.5	42.1
l-methylphenanthrene	0.33 ± 0.02	0.77:0.15	0.24 ± 0.02	0.3130.04	+133.3	27.3	6.7
2-methylphenanthrene	0.21 ± 0.01	0.53 ± 0.04	0.1930.01	0.22 ± 0.02	+152.4	9.5	4.8
3,6-dimethylphenanthrene	0.11 ± 0.00	0.53 ± 0.04	0.24 ± 0.05	0.20:0.04	+381.8	+118.2	+81.8
Total (naphthalene - 3,6-dimethylphenanthrene)	9.9130.15	6.21 ± 0.42	6.27:0.41	9.0331.05			

[a] Monoaromatic hydrocarbons have not been corrected for recovery.

[b] % decrease = $\frac{\text{Concentration in original oil - concentration in weathered oil found}}{\text{concentration in original oil}}$ X 100; (+) sign indicates higher concentration of compound in weathered oil on weathered oil basis as compared to-original oil.

[c] Compound not detected at the sensitivity level that analyses were conducted.

‘Relative decrease in concentration % was not calculated because recovery data was not obtained on monoaromatic hydrocarbons.

are more graphically depicted in Figure 2 where the gas capillary chromatogram of the aromatics fraction of the original crude oil is compared to the aromatics fraction derived from the 24-day weathered oil sample of tank #1.

Confidence limits were established and significant differences determined for various saturate and aromatic hydrocarbons and hydrocarbon types in the three exposure systems. These results are compiled in Table 5. Variability associated with the sum of all saturate hydrocarbons C_{12}-C_{26} including pristane and phytane indicated differences between tank #1 vs. tank #2 and #3 to be significant; however, total differences between tanks #2 and #3 were not significant. Significant differences were also found for the concentrations of all aromatic hydrocarbons and hydrocarbon types (naphthalene - 3,6-dimethylnaphthalene) between tanks #1 and #3. Significant trends in the differences in concentrations of the aromatic components of tank #1 vs. tank #2 and tank #2 vs. tank #3 are more subtle. No significant differences in the concentrations of diaromatic hydrocarbons and hydrocarbon types are observed between tanks #1 and #2; however, significant differences are observed for the triaromatics. Although significant differences in the concentrations of naphthalene, methylnaphthalenes, and phenanthrene were observed, no trends were observed for the aromatic components of tanks #2 vs. #3.

DISCUSSION

The combination of light and water exposure parameters of Tank #1 produced the largest relative decreases in volatile saturate (C_{12} to C_{26}) and most aromatic (naphthalene -2, 3, 6-trimethylnaphthalene) -hydrocarbons relative to the original oil.

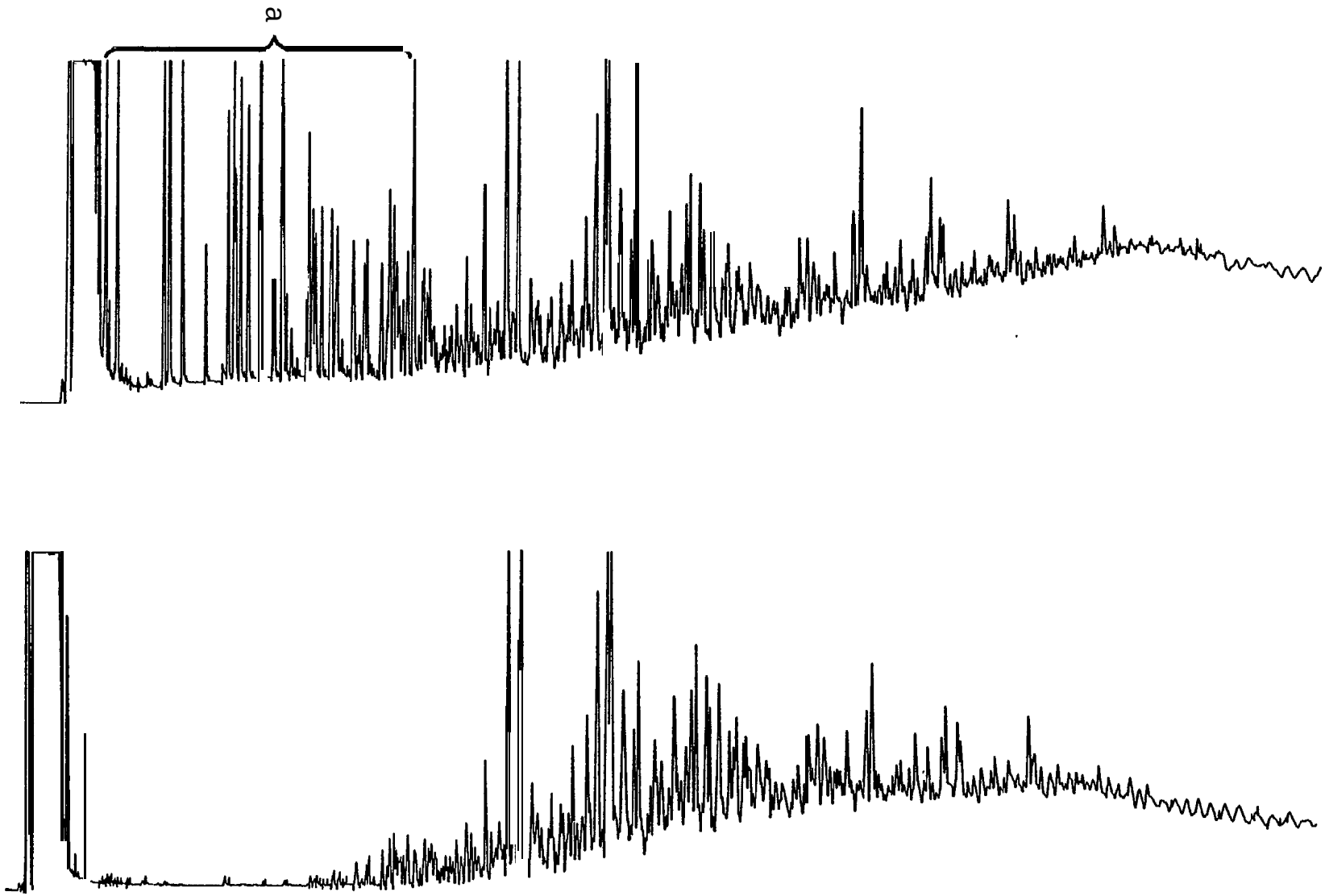

FIGURE 2. Gas capillary chromatograms of aromatic hydrocarbon fraction from original Prudhoe Bay Crude Oil (top) and aromatic hydrocarbon fraction from 24-day weathered oil sample from Tank #1 (bottom). A denotes monoaromatic hydrocarbon region.

TABLE 5 SIGNIFICANCE IN THE RELATIONSH1PS BETWEEN THE CONCENTRATIONS OF VARIOUS SATURATE AND AROMATIC HYDROCARBONS AND HYDROCARBON TYPES AND THE TYPE OF EXPOSURE. CONFIDENCE LIMITS ARE MEASURED AT THE 95% LEVEL. S.D. = SIGNIFICANTLY OIFFERENT, N.S.D. = NOT SIGNIFICANTLY DIFFERENT.

Hydrocarbon or Hydrocarbon Type	Concentration in mg/gram Oil		
	Tank #1	Tank #2	Tank #3
$(C_{12} - C_{26})$ + Pristane + Phytane	7.30 ± 0.10	21.98 ± 0.61	23.98 ± 4.27
		S.D.	S.D. N.S.D.
Naphthalene	0.06 ± 0.01	0.16 ± 0.01	0.52 ± 0.08
		S.D.	N.S.D. S.D.
Methylnaphthalenes	1.01 ± 0.12	1.43 ± 0.14	2.54 ± 0.34
		S.D.	N.S.D S.D.
Dimethylnaphthalenes	2.29 ± 0.22	2.83 ± 0.27	4.12 ± 0.54
		S.D.	N.S.D. N.S.D
2,3,6-Trimethyl-naphthalene	0.26 ± 0.03	0.39:0.12	0.43:0.04
		S.D.	N.S.D. N.S.D.
Phenanthrene	0.61 ± 0.06	0.34 ± 0.05	0.22 ± 0.01
		S.D.	S.D. S.D.
Methylphenanthrenes	1.19 ± 0.06	0.43 ± 0.03	0.52 ± 0.05
		S.D.	S.D. N.S.D.
3,6-Dimethylphenanthrene	0.53 ± 0.04	0.26 ± 0.03	0.20 ± 0.04
		S.D.	S.D. N.S.D.
Total Aromatics (Naphthalene - 3,6-dimethylphenanthrene)	6.21 ± 0.42	6.27 ± 0.41	9.03 ± 1.05
		S.D.	N.S.D. N.S.D.

Significant changes have been reported in the ratios of nC_{17}/pristane and nC_{18}/phytane after 6 to 9 months of exposure of oil contaminated intertidal sediments in field recruitment studies (Anderson et al., 1978) which suggested the presence of hydrocarbon utilizing organisms (Blumer & Sass, 1972). No changes were observed in these ratios in weathered oil from these three exposure systems. These data suggest that biodegradation was a minor process in shaping the composition of the weathered oils produced in these exposure systems in this short period of time. Detectable amounts of monoaromatic hydrocarbons were absent in all three weathered oils as were the saturate hydrocarbons from C_8 to C_{10}. Similar, but qualitative results have been reported from the dissolution of #2 fuel oil in laboratory studies (Zucher et al., 1978). The different exposure parameters of Tank #1 and #3 -produced the greatest differences in the volatile hydrocarbon content of these oils following 24 days of weathering. The decreases in the content of most aromatic compounds were less for oil protected from light, water agitation and somewhat from air circulation (Tank #3). The sun shield reduced air flow over the oil and the only component shown to increase somewhat in Tank #3 in proportion to the original oil was the heaviest compound, 3, 6-dimethyl-phenanthrene. This component was enriched to a greater extent in Tank #2 and in Tank #1 its contribution to the total was nearly four times as great. There was an increasing degree of enrichment in the weathered (mousse) oil in Tank #1 as molecular weight increased from phenanthrene to the 3, 6-dimethylphenanthrene. These data may well indicate that higher molecular weight compounds of 4 and 5 rings (polynuclear aromatics) are also enriched in weathered oil, perhaps relative to their molecular weight. This is of

considerable significance, since tissue contamination or effects from these potential carcinogenic or mutagenic compounds could be enhanced by the weathering processes. The polynuclear aromatics are less water soluble than those identified in Table 4, so it is not likely that water column species would be affected. However, the mixing of weathered oil with sediments may produce an environment more hazardous to benthic species than contamination from fresh oil. Oil in sediments containing a higher proportion of higher molecular weight compounds, would appear to have greater potential for tissue contamination (Roesijadi et al., 1978), but the effects of such exposures can not be estimated at this time. To determine the actual significance of the weathered oil to marine organisms, field experiments should be conducted and data on accumulation and effects compared to fresh oil exposures.

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Brand names and manufacturers are cited to assist the reader in replication of the experiments, however, use does not constitute endorsement by Battelle Memorial Institute.

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VI. DISTRIBUTION AND FATE OF POLYAROMATIC HYDROCARBONS IN SEDIMENT-WATER SYSTEMS

VI-A:

DISTRIBUTION AND FATE OF POLYAROMATIC HYDROCARBONS IN INTERSTITIAL WATER AND SEDIMENT

SUMMARY OF RESULTS

One day after ^{14}C-labelled phenanthrene was added to an oil-contaminated coarse-grained sediment in a simulated intertidal exposure system, about ½ of 1 percent of the radiolabel was present in the interstitial water. The remainder was associated with the sediment, presumably in a film of oil surrounding the individual particles. The proportion of radiolabel in the water gradually increased, reaching 3% after 60 days. The proportion of radiolabel arising from chrysene and benzo(a)pyrene contained in the water was lower by 1 to 3 orders of magnitude. It ranged from 0.05% to 0.1% for chrysene and 0.01 to 0.04% for benzo(a)pyrene after 1 and 60 days, respectively. When ^{14}C-labelled phenanthrene was added to fine-grained sediment in a similar system, only 0.1% of the label appeared in interstitial water after 1 day, 0.53% after a week and 0.16% after 60 days. The proportion of label from chrysene and benzo(a)pyrene in interstitial water was the same in fine as in coarse-grained sediment.

The labelled compounds found in interstitial water from fine-grained sediment exposed to ^{14}C-phenanthrene were further characterized. After 7 days of exposure only 15% of the label was present as parent phenanthrene or moderately polar degradation products. After 60 days this proportion was reduced to 1.5%. $^{14}CO_2$ comprised approximately 35%, 45%, and 72% of the labelled products after 7, 30, and 60 days, respectively.

The time course of parent compound and CO_2 concentrations are consistent with a gradual degradation of phenanthrene by way of intermediate metabolic products. The metabolizes present in interstitial water from fine-grained sediment after 7, 15, and 30 days exposure to label led phenanthrene-were separated by high-pressure liquid chromatography. Three to four different products were present at each time period. The retention times of the major products were approximately 14.5, 16.0, and 4.5 minutes. Conventional microbiological cultures of marine sediments containing ^{14}C-phenanthrene produced metabolizes with the same retention times as those found in the simulated intertidal system, though in different proportions. This finding suggests that the degradative processes observed in controlled laboratory experiments are valid models of the processes occurring under natural environmental conditions.

VII . APPENDIX

PUBLICATIONS RESULTING FROM RESEARCH CONDUCTED ON THE NOAA/BLM OCSEAP PROGRAM UNIT 454

by the Staff of

Battelle Pacific Northwest Laboratories

PUBLICATIONS RESULTING FROM RESEARCH
CONDUCTED ON THE NOAA/BLM OCSEAP PROGRAM UNIT 454

by the staff of

BATTELLE PACIFIC NORTHWEST LABORATORIES

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Anderson, J. W., G. Roesijadi, D. L. Woodruff and E. A. Crecelius. 1977. Research to Determine the-Accumulation of Organic Constituents and Heavy Metals from Petroleum-impacted Sediments by Marine Detritivores of the Alaskan Outer Continental Shelf. Annual Report to NOAA, Boulder, CO., April, 1977, pp. 47.

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